

RAD HRVATSKE AKADEMIJE ZNANOSTI I UMJETNOSTI
KNJIGA 499

R A D

CROATIAN ACADEMY
OF SCIENCES AND ARTS

499

MEDICAL SCIENCES

32



ZAGREB, 2008.

R A D

HRVATSKE AKADEMIJE
ZNANOSTI I UMJETNOSTI

499

MEDICINSKE ZNANOSTI

32



ZAGREB, 2008.

HRVATSKA AKADEMIJA ZNANOSTI I UMJETNOSTI
RAZRED ZA MEDICINSKE ZNANOSTI
CROATIAN ACADEMY OF SCIENCES AND ARTS
DEPARTMENT OF MEDICAL SCIENCES

Glavni i odgovorni urednik / Editor-in-Chief

Marko Pečina

Urednički odbor / Editorial Board

Ivana Čepelak

Vida Demarin

Vjekoslav Jerolimov

Josip Madić

Aleksandra Mišak

CONTENTS

<i>Marko Pećina</i> Editorial	7
<i>Mario Jug, Mira Bećirević-Lačan</i> Cyclodextrin-Based Pharmaceuticals (REVIEW)	9
Symposium 55 YEARS FROM ORGANIZING THE BEGINNING OF ALLERGOLOGY IN CROATIA: FUTURE WE BUILT ON THE PAST	27
<i>Karl Holubar</i> Salomon Stricker (1834-1898): A Pioneer Micro-biologist (REVIEW)	29
<i>Stella Fatović-Ferenčić</i> Beginnings and Development of Allergology in Croatia from 1870 to 1970 (REVIEW)	35
<i>Jasna Lipozenčić, †Albin Brnobić</i> Academician Franjo Kogoj and Allergology in Croatia (REVIEW)	53
<i>Alenka Gagro</i> Novel Concepts in Immunology and their Application in Clinical Allergology (REVIEW)	67
<i>Jasna Lipozenčić, Suzana Ljubojević</i> Atopic Dermatitis (REVIEW)	77
<i>Asja Stipić-Marković, Branimir Čvorišćec, Branko Pevec, Mira Radulović-Pevec</i> Increasing Incidence of Allergy in Croatia (REVIEW)	105
<i>Slavica Dodig</i> Current Laboratory Diagnosis of Allergy (REVIEW)	117
<i>Book Reviews</i> <i>Željko Dugac</i> Karl Holubar, Stella Fatović-Ferenčić, Gerd Plewig: Looking at Eyes and Faces. Ophthalmologic Water-colours Drawn Largely by Physician-artists Vienna, late 18 th and 19 th century.	129

SADRŽAJ

Marko Pečina Uvodnik	7
Mario Jug, Mira Bečirević-Lačan Ciklodekstrini u ljeikovitim oblicima (PREGLEDNI RAD)	9
Simpozij 55 GODINA OD ORGANIZIRANIH POČETAKA ALERGOLOGIJE U HRVATSKOJ: BUDUĆNOST GRADIMO NA PROŠLOSTI	27
Karl Holubar Salomon Stricker (1834-1898): Pionir mikro-biolog (PREGLEDNI RAD)	29
Stella Fatović-Ferenčić Početak i razvoj alergologije u Hrvatskoj u razdoblju od 1870. do 1970. godine (PREGLEDNI RAD)	35
Jasna Lipozenčić, †Albin Brnobić Akademik Franjo Kogoj i alergologija u Hrvatskoj (PREGLEDNI RAD)	53
Alenka Gagro Suvremene spoznaje u imonologiji i njihova primjena u kliničkoj alergologiji (PREGLEDNI RAD)	67
Jasna Lipozenčić, Suzana Ljubojević Atopijski dermatitis (PREGLEDNI RAD)	77
Asja Stipić-Marković, Branimir Čvorišćec, Branko Pevec, Mira Radulović-Pevec Povećana incidencija alergija u Hrvatskoj (PREGLEDNI RAD)	105
Slavica Dodig Suvremena laboratorijska dijagnostika alergije (PREGLEDNI RAD)	117
Prikazi knjiga Željko Dugac Karl Holubar, Stella Fatović-Ferenčić, Gerd Plewig: Looking at Eyes and Faces. Ophthalmologic Water-colours Drawn Largely by Physician-artists Vienna, late 18 th and 19 th century.	129

EDITORIAL

For any journal, the continuity is one of the most important proofs of both its good name and its credibility. It is therefore with great pleasure that I present to you the third volume of our journal, which has been continually published since 2006 (RAD 30, 2006; RAD 31, 2007, and now RAD 32, 2008). I proudly announce to you that, at the website <http://hrcak.srce.hr>, RAD 30 and RAD 31 have been visited by a significant number of interested colleagues (more than 3,500 in 2007). Moreover, it is a pleasure for me to inform you that RAD 31, 2007 has become available at libraries worldwide via EBSCO Publishing's databases. Our next step is to enter SCOPUS and thereby join the family of 15,000 peer-reviewed journals from more than 4,000 publishers.

In the issue in front of you, you can find the papers from the symposium entitled *55 YEARS FROM ORGANIZING THE BEGINNING OF ALLERGOLOGY IN CROATIA: FUTURE WE BUILT ON THE PAST*, which was held in October 2007. In this context, I would primarily like to thank Mr. Dragan Dekaris, full member of the Croatian Academy of Sciences and Arts, and Prof. Jasna Lipozenčić, M.D. Ph.D., for their assistance regarding the publishing of the said papers. My sincere thanks are furthermore directed to all the authors, not only for respecting the deadline for submitting the papers, but also for responding without delay to the suggestions of the reviewers, to whom I also express my thanks for having done their job thoroughly and promptly.

As I have already mentioned in the Editorial to RAD 31, we shall endeavour to enhance the quality of the papers published and to enter the globally relevant databases. In this process, we expect a continued support not only from the members of the Department of Medical Sciences of the Croatian Academy of Sciences and Arts, but also from the entire biomedical community.

Marko Pećina

CYCLODEXTRIN-BASED PHARMACEUTICALS

Mario Jug*, Mira Bećirević-Laćan

Department of Pharmaceutics, Faculty of Pharmacy and Biochemistry,
University of Zagreb

Summary

Cyclodextrins, cyclic oligosaccharides, are useful pharmaceutical excipients, which have attracted widespread attention. The ground for their popularity from a pharmaceutical point of view is in their ability to change undesirable characteristics of the drugs, such as unpleasant taste, low aqueous solubility, chemical instability and improving the drug bioavailability by inclusion complex formation. Due to their biocompatibility, cyclodextrins may be used in formulations intended for different routes of drug application including oral, ocular, nasal and even parenteral route. This article outlines the importance and functionality of cyclodextrin in different dosage forms and indicates the possible directions of future development.

Keywords: cyclodextrin; inclusion complex; drug delivery; aqueous solubility; chemical stability; bioavailability

INTRODUCTION

The main goal of pharmaceutical research is the development of new and improved drugs with fewer side effects. Over the years drug discovery has evolved to the point that rational drug design and high-throughput screening techniques have become routine. Through this processes it is possible to rapidly identify active compounds. But many of new drug molecules have failed *in vivo*. Retrospective studies have shown that more than 40 % of new drug failures in development can be connected with poor biopharmaceutical properties of the drug molecule, such as low solubility and/or poor drug permeability across the biomembranes in the body. For a drug molecule to be successfully delivered to the site of action *in vivo*, the drug must possess sufficient aqueous solubility. This allows efficient drug dissolution and delivery of dissolved drug molecule

* Correspondence author: Dr. sc. Mario Jug, Department of Pharmaceutics Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10 000 Zagreb, E-mail: mjug@pharma.hr

to the mucosal surface of the body. At the same time, the drug must possess sufficient lipophilicity to be able to pass through lipophilic mucosal membrane of the body. Various formulation techniques have been developed in an effort to overcome these and other obstacles in sufficient drug delivery. One of approaches may be in the use of drug-cyclodextrin inclusion complexes [1, 2]. Because of their multifunctional characteristics and bioadaptability, cyclodextrins are capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. Thus, cyclodextrins have important role in development of new drugs, as well as in optimisation of pharmaco-therapeutic properties of already known drugs.

The aim of this article is to give an insight into physicochemical properties of cyclodextrins and their inclusion complexes with emphasis on their current pharmaceutical applications. In addition, other possible application and future trends in development of cyclodextrin-based drug formulations will be discussed.

CHEMICAL STRUCTURE OF CYCLODEXTRINS

Cyclodextrins are a group of structurally related cyclic oligosaccharides produced by bacterial starch degradation [3]. Naturally occurring cyclodextrins (α -, β - and γ -cyclodextrin, respectively) are consisting of 6, 7 or 8 α -[1,4] linked D-glucopyranose units. Cyclodextrins with less than 6 glucopyranose units cannot be formed due to sterical reasons, while cyclodextrins with more than 8 glucopyranose units (so called large cyclodextrins) are described in the literature, but their pharmaceutical importance is still under investigation [4]. Because of the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone (Figure 1). The hydroxyl groups are orientated to the exterior surface of the molecule providing hydrophilic properties.

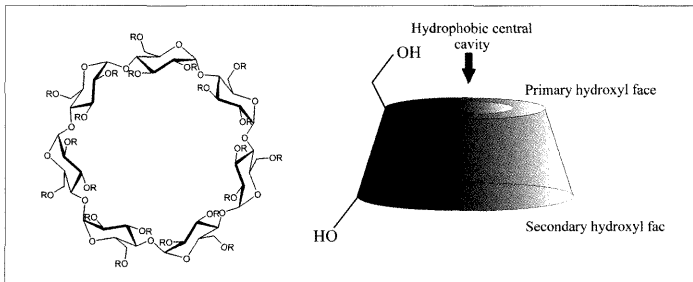


Figure 1. The chemical structure of β -cyclodextrin ($R=H$).

Table 1. The physicochemical properties of some pharmaceutically important cyclodextrin derivatives.

CYCLODEXTRIN	N ^(a)	R	Substitution ^(b)	MW ^(c)	Solubility in water (mg mL ⁻¹) ^(d)
α -cyclodextrin	6	-H	-	972	145
β -cyclodextrin	7	-H	-	1135	18.5
2-hydroxypropyl- β -cyclodextrin	7	-CH ₂ CHOHCH ₃	0.65	1400	> 600
sulfobutylether- β -cyclodextrin sodium salt	7	-(CH ₂) ₄ SO ₃ ⁻ Na ⁺	1.8	1312	> 500
randomly methylated- β -cyclodextrin	7	-CH ₃	0.9	2163	> 500
γ -cyclodextrin	8	-H	-	1297	80

(a) Number of glucopyranose units

(b) Average number of substituents per glucopyranose unit

(c) Molecular weight (MW) in Daltons

(d) Solubility in pure water at 25°C

Due to lack of the rotation around a-[1,4] bonds, the primary hydroxyl groups are orientated to the narrow edge of the cone, while the secondary groups are at wider edge of the molecule. The central cavity of the molecule is lined with skeletal carbon and etheral oxygen atoms of the glucopyranose units, which gives it a lipophilic character.

Due to strong bonding of the cyclodextrin molecules in the crystal state, the water solubility of natural cyclodextrins is much lower compared to the corresponding linear dextrins. Also, the formation of intermolecular hydrogen bonds between 2- and 3-hydroxyl group of the adjacent glucopyranose units diminish the ability of cyclodextrins to form hydrogen bonds with the surrounding water molecules. This is especially pronounced in case of β -cyclodextrin, where the belt consisted of 7 hydrogen bonds was formed, limiting its solubility (Table 1). In case of α -cyclodextrin only 5 of possible 6 hydrogen bonds are formed due to sterical reasons, while γ -cyclodextrin hydrogen has noncoplanar and more flexible structure. Therefore, the water solubility of α - and γ -cyclodextrin are 7.8 and 11.2 times higher than that of β -cyclodextrin. The substitution of any hydroxyl group involved in hydrogen bond formation, even with lipophilic moiety such as methyl group, results in disruption of intermolecular hydrogen bonds that dramatically increase the cyclodextrin solubility in water. The number of possible isomers generated by random substitution of β -cyclodextrin is large, since it has 7 primary and 14 secondary hydroxyl groups in the structure. This contributes to the transition of the crystalline β -cyclodextrin to amorphous compound in case of its derivatives, additionally contributing to the water solubility. Till now, more than 1500 different cyclodextrin derivatives have been synthesized and described in the literature [2, 3, 5]. Some hydrophilic

cyclodextrin derivatives of pharmaceutical importance are listed in Table 1. Other derivatives include lipophilic derivatives (alkylated and acetylated cyclodextrins), cyclodextrins with pH dependent solubility (carboxymethyl- β -cyclodextrin) and different cyclodextrin based polymers [3].

TOXICOLOGICAL AND REGULATORY CONSIDERATIONS

Safety is a primary concern when considering new excipients intended for use in pharmaceutical formulations. Therefore, toxicological issues and biological fates of cyclodextrins have been thoroughly investigated. Cyclodextrins are resistant to the degradation by human salivary and pancreatic amylases. Due to high molecular weight and hydrophilic nature ($\log P_{ow}$ between -3 and 0), natural cyclodextrins permeate the lipophilic biological membranes with considerable difficulties. Therefore, only 1-3% of applied cyclodextrin dose is absorbed in gastrointestinal tract, while dermal, ocular and nasal bioavailability is even lower. The absorbed cyclodextrins are excreted intact via the kidney. The bulk of orally administered cyclodextrins is metabolised in colon, by the colon micro-flora to primary metabolites, such as acyclic maltodextrins, maltose and glucose. After absorption to the systemic circulation, primary metabolites are further metabolised and finally excreted as CO_2 and H_2O [3, 6, 7].

The toxicological studies have demonstrated that orally administered cyclodextrins are practically non-toxic, due to lack of absorption from the gastrointestinal tract. Furthermore, a number of safety evaluations have shown that γ -cyclodextrin, hydroxypropyl- β -cyclodextrin and sulphobutylether- β -cyclodextrin appear to be safe *even when administered parenterally*. β -cyclodextrins form complexes with cholesterol that accumulate in the kidney and produce renal tubule damage, while α -cyclodextrin and methylated- β -cyclodextrins produce dose dependent haemolysis. Therefore, they are not suitable for parenteral administration [1,6].

Cyclodextrins are relatively new from a regulatory point of view and policies on their use are still not standardised. The regulatory status of cyclodextrins is continuously evolving and current state is presented in Table 2. Consensus seems to be building among regulators that cyclodextrins are excipients and not a part of the drug substance, which is logical based on their physicochemical properties as drug solubilizers and stabilizers [7].

INCLUSION COMPLEX FORMATION

In aqueous solution, cyclodextrins are able to form inclusion complexes with many drugs by incorporating the drug molecule, or more often a lipophilic moiety of the drug molecule into central cavity (Figure 2.). This process may be explained as drug

Table 2. The regulatory status of some pharmaceutically important cyclodextrins.

Cyclodextrin	Pharmacopoeia		
	PH. EUR. ^(a)	USP/NF ^(b)	JPC ^(c)
α -cyclodextrin	Yes	No	Yes
β -cyclodextrin	Yes	Yes	Yes
2-hydroxypropyl- β -cyclodextrin	Yes	Yes	No
sulfobutylether- β -cyclodextrin sodium salt	No	No	No
randomly methylated- β -cyclodextrin	No	No	No
γ -cyclodextrin	In progress	Yes	Yes

(a) European Pharmacopoeia 5th Edition (2005)

(b) United States Pharmacopoeia 28th Edition/National Formulary 23rd Edition (2005)

(c) Japanese Pharmaceutical Codex (1997)

encapsulation at the molecular level. The main driving force leading to the inclusion complex formation is in the release of enthalpy rich water molecules from the central cavity of the cyclodextrin molecule. No covalent bonds are formed or broken during the inclusion complex formation. Electrostatic, van der Waals or hydrophobic interactions and hydrogen bonding between included drug moiety and cyclodextrin molecule are leading to the conformational strain release of cyclodextrin molecule, additionally contributing to the inclusion complex formation. Since the drug/cyclodextrin interactions are relatively weak, drug molecules included in the cyclodextrin cavity are in rapid equilibrium with the free drug molecules in the solution [3, 5].

Cyclodextrins will form the inclusion complexes with drug molecules that have compatible geometry and are less polar than water. Most drug molecules will form 1:1 complexes with cyclodextrins, but the 1:2 and higher order complex formation have

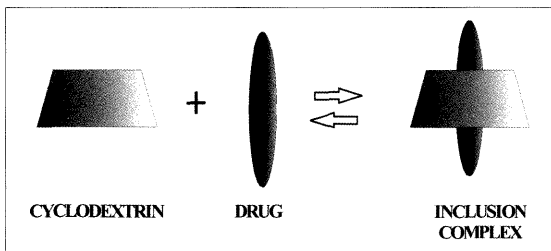


Figure 2. Schematic illustration of the inclusion complex formation.

been reported. Inclusion complex formation will change the physiochemical properties of the drug, such as its solubility and chemical stability. The cyclodextrin molecule forms a hydrophilic shield around encapsulated lipophilic moiety of the drug, increasing the apparent solubility of the drug. Also, inclusion complex formation may protect chemically labile drug molecules from the surrounding environment, thereby reducing or even preventing drug hydrolysis, oxidation, racemization and enzymatic decomposition. Therefore, cyclodextrins are very useful pharmaceutical excipients suitable for the development of different drug formulations. The list of some available drug formulations containing cyclodextrins is presented in Table 3 [3, 5, 8].

CYCLODEXTRINS IN DRUG FORMULATIONS

Stability issues may limit the feasibility of a pharmaceutical formulation. This is especially pronounced in case of aqueous drug formulation, due to potential hydrolysis or oxidation of an active compound. Many studies have shown that stability of chemically labile drugs can be improved through complexation with cyclodextrins. Very illustrative example is in the stabilising effect of cyclodextrins on different prostaglandins that allowed the development of suitable formulations [3]. One of these compounds, PGE_2 possesses potent oxytocin-like effect and was of interest as a possible agent for the labour induction in childbirth. The high instability of this agent complicated the development of a suitable formulation. Inclusion complex formation using β -cyclodextrin resulted in a significant increase of the PGE_2 stability in solid state. Cyclodextrin based PGE_2 sublingual formulation (Prostarmon E; Ono) was approved for Japanese market in 1976. Prostarmon E is highly effective and represents a significant medical advance, especially for the labour induction in oxytocin-insensitive individuals, but also for its tendency to produce less bleeding after delivery. Another prostaglandin, PGE_1 relaxes smooth muscles and increases the blood flow. This compound might be used in treatment of peripheral circulatory disorders, but the clinical utility of PGE_1 was limited by its metabolic degradation. The stabilising effect of α -cyclodextrin on PGE_1 allowed the development of parenteral formulation (Prostavasin) that showed activity against chronic arterial occlusion and arteriosclerosis. Also, PGE_1/α -cyclodextrin formulation was effective in treatment of male erectile dysfunction [9]. Intracavernous application of the complex was found to be effective in subjects who were not responsive to sildenafil citrate (Viagra®, Pfizer).

Another goal that might be obtained by inclusion complex formation is the reduction of unpleasant drug's taste or smell [8,10]. The adstringent and irritating effect of nicotine excluded its direct consumption in the form of sublingual tablets or chewing gums. This problem was successfully solved by inclusion complex formation using β -cyclodextrin. The molecular encapsulation prevented the direct contact of nicotine with the

taste buds in the oral cavity, allowing the formulation of sublingual tablets for smoking cessation (Nicorette Microtab®). Another similar example is cetizirine hydrochloride, an antihistaminic drug used for allergic symptoms relief. Inclusion complex formation with β -cyclodextrin reduced very bitter taste of this drug, allowing the formulation of a chewing tablet. This kind of formulation provided easy consumption of the drug and relief of allergic symptoms in situation when water is not available (e. g. during driving).

The most common pharmaceutical application of cyclodextrin is their use as solubilizers [5]. In ophthalmology, the local drug administration in form of aqueous solutions drops is preferred. The development of suitable formulation may be considerably hampered by drug solubility, since the drug dose must be dissolved in 0.03-0.05 mL of aqueous solution [12]. The solubilizing effect of cyclodextrin would allow the incorporation of the drug dose into volume suitable for ocular application. Cyclodextrins are proved to be useful excipients in eye drop formulations of various drugs including different corticosteroids, carbonic anhydrase inhibitors, pilocarpine, cyclosporins, diclophenac, etc. It is likely that the use of cyclodextrins in topical delivery to the eye will expand the selection of drugs available for the use in ophthalmology [13]. Isotonic drug solutions may be prepared by using up to 20% (w/v) concentration of hydroxypropyl- or sulphobutylether- β -cyclodextrin at physiological pH-values. Addition of water-soluble polymers to the aqueous cyclodextrin solution would further increase the drug solubility due to ternary drug-cyclodextrin-polymer complex formation [5, 14]. Also, the cyclodextrins have been used to reduce the ophthalmic drug irritation and to increase the drug stability in the ophthalmic formulations. The drawback of the cyclodextrin use in ophthalmic formulation is in the fact that they may interact with the preservatives in the formulation, reducing their efficiency [12]. The solution of this problem might be in the use of a novel cyclodextrin derivate containing quaternary amino groups. This cyclodextrin derivate has been shown to be a multifunctional agent having the effect on the drug solubility and acting as antibacterial agent [5].

The cyclodextrin drug solubilization was successfully applied in development of formulations aimed for systemic drug delivery via the nasal mucosa (15). The large absorptive surface and high vascularity of the nasal mucosa ensure a rapid absorption of compounds under circumvention of the hepatic first pass elimination. The pharmacokinetic drug profile after nasal application is often similar to that of parenteral application. The easy accessibility of the nasal route facilitates self-medication, thus improving patient compliance when compared to parenteral route [16]. Randomly methylated- β -cyclodextrin was successfully applied in development of nasal formulation aimed for pulsed oestrogen therapy in treatment of climacteric symptoms (Aerodiol). The nasal absorption of oestrogen from cyclodextrin-based formulation was rapid and peak plasma concentrations were achieved within 10-30 minutes after application. The oestrogen

plasma levels returned to baseline within 2 hours. This pattern is more akin to physiological oestrogen secretion than that associated with oral or transdermal application. Also, oral application of oestrogen generated high oestrone/oestradiol ratio, while nasal delivery gave rise to more physiological ratio of those hormones [17,18]. Randomly methylated- β -cyclodextrin was also used in development of dihydroergotamine nasal formulation aimed for migraine treatment. The benefit of nasal drug application in this case is in fact that migraine attacks are usually accompanied by vomiting therefore oral drug application is inadequate [19]. Numerous studies have demonstrated that cyclodextrins are efficient nasal permeation enhancers for different drugs, including hormones and peptides [15]. The cell-culture based studies indicated that randomly methylated- β -cyclodextrin might open the tight junctions in the nasal mucosa triggering paracellular drug absorption. The possibility of using the cyclodextrins as nasal absorption enhancer is currently under investigation. Histological and cell-culture based studies [20] showed that cyclodextrins did not induce mucosal damage and had no significant influence on the nasal ciliary movement [21]. Further, clinical data [15] showed no significant adverse effect, indicating that cyclodextrins are effective and safe excipients in nasal drug delivery.

In solid state, cyclodextrins convert the drug of interest, which is usually crystalline, into a dispersion of the amorphous drug in the cyclodextrin carrier. Importantly, the dispersion is a result of a molecular encapsulation by cyclodextrins [3]. Improved complex solubility and amorphous drug state will increase the drug dissolution rate and generally will enhance the oral bioavailability of the drugs with low water solubility. After dissolution of the inclusion complex in gastrointestinal tract, cyclodextrins are acting as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane, where drug permeation across the membrane occurred. The relatively lipophilic gastrointestinal membrane has low affinity for the hydrophobic cyclodextrin molecule and they remain in the aqueous membrane exterior. Numerous studies have confirmed the increased oral bioavailability and faster onset of action for many drugs upon cyclodextrin complex formation, including carbamazepine, digoxin, furosemide, glibenclamide, itraconazole, ketoprofen, nifedipine, spirinolactone, etc. In the same time, cyclodextrins may reduce some side effects of orally administered drugs [2,8,22].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of painful and inflammatory conditions, but they chronic application may result in upper gastrointestinal tract irritation and even bleeding. The very illustrative example is piroxicam, the drug useful in the treatment of osteoarthritis and rheumatoid arthritis, as well as gout, acute musculoskeletal disorders and dysmenorrhoea. The relatively long pharmacokinetic half-life of the drug allows the possibility of once a day dosing in contrast to many other NSAIDs, but poor water solubility leads to the slow onset of

action. The inclusion complex formation using β -cyclodextrin increased the drug solubility more than fivefold. The enhanced drug solubility leads to the increased drug dissolution rate and faster absorption rate. This resulted in higher piroxicam plasma levels at early time after application to humans and consecutive faster onset of drug action. Several endoscopic studies as well as a research using adhesive radio-substrates have revealed significant reduction of gastroduodenal damage after administration of piroxicam- β -cyclodextrin complex to humans [1,23]. Therefore, several branded piroxicam- β -cyclodextrin based formulations are available worldwide (Table 3).

The limitation in the use of cyclodextrins in oral drug administration is in the effect of cyclodextrins on the formulation bulk. Incorporation of cyclodextrins into solid dosage forms will increase the formulation bulk at least 4-fold. Therefore, the cyclodextrins can be used only in solid dosage formulation of relatively potent drugs with the single dose up to 100 mg. In other cases, the formulation size would be too large, and swallowing the tablet would not be possible [22].

Taking into consideration the numerous advantages of the cyclodextrin complexation of the drugs, it is not evident why the number of approved and marketed cyclodextrin based formulation of long known (generic) drug is relatively low. The price and approval status of the cyclodextrins is no more a restricting factor for their use. The crucial problem is in fact that for approval of any new formulation of already known (generic) drug, a bioequivalence test has to be performed. A cyclodextrin-based formulation is practically never bioequivalent with the reference product (the earlier approved formulation), but significantly superior in terms of improved solubility, faster and more complete absorption that leads to enhanced biological activity of the drug. Therefore, cyclodextrin based drug formulation will not be a simple generic, but a supgeneric drug. In this case, the authorities are requesting the repetition of the largest part of long lasting and very costly clinical studies. Therefore, the costs of development will be nearly as high as in case of an original drug. In the same time, the market for cyclodextrin-based formulation will be considerably narrower. Taking into account that the absorption of the drug in reduced dose from cyclodextrin-based formulation is faster than from its original formulation, then a simple Clinical I phase should be satisfying from regulatory point of view. All deviation between the pharmacological effect of the original formulation and cyclodextrin-based formulation is the consequence of quicker and more complete absorption of the last one. No further change in pharmacodynamic or therapeutic effect of the drug might arise in consequence of the cyclodextrin complexation [8]. Taking into account the superior therapeutic effect of cyclodextrin based formulation, such as reduced drug dose that may decrease the number and intensity of the side effects as well as faster onset of drug action, it might be expected that in the near future the number of cyclodextrin based formulations on the market will grow.

Table 3. Some of marketed drug formulations containing cyclodextrin.

Drug	Trade name	Indication	Formulation	Company (country)
a-cyclodextrin				
Alprostadi (PGE ₁)	Prostavasin Caverject	Chronic arterial occlusive disease	Intravenous solution	Ono (Japan) Pfizer (Europe)
Cefotiam hexetil HCl	Pansporin T	Antibiotic	Oral tablet	Takeda (Japan)
Limaprost	Opalmon, Prorenal	Buerger's disease	Oral tablet	Ono (Japan)
PGE ₁	Edex	Erectile dysfunction	Intracavern injection	Schwartz Pharma (G)
b-cyclodextrin				
Benxate	Ulgut Lonmiel	Antiulcerant	Oral capsule	Teikoku (Japan) Shionogi (Japan)
Dexamethasone	Glymesason	Analgesic, antiinflammatory	Dermal ointment	Fujinaga (Japan)
Dinoprostone (PGE ₂)	Prostarmon E	Induction of labour	Sublingual tablet	Ono (Japan)
Diphenhydramine/Chlorteophilin	Stada-Travel	Travel sickness	Chewing tablet	Stada (Europe)
Nicotine	Nicorette Microtab Nicogum	Smoking cessation	Sublingual tablet, chewing gum	Pharmacia (Sweden) Pierre Fabre (France)
Nimesulide	Nimedex Mesulid Fast	NSAID	Oral tablet, oral sachet	Novartis (Europe)
Nitroglycerin	Nitrophen	Coronary dilator	Sublingual tablet	Nippon Kayaju (Japan)
Omeprazole	Omebeta	Proton pump inhibitor	Gastro resistant capsule	Betapharm (Germany)
Piroxicam	Brexin Cicladol Flogene	Analgesic, antiinflammatory	Oral tablet, suppository	Bipharma i Hermes i Pharmafontana (Hui) Ache (Brazil)
Tiaprofenic acid	Surgamyl	Analgesic	Oral tablet	Roussel-Maestrelli (France)
hydroxypropyl-b-cyclodextrin				
Cisapride	Propulsid	Gastrointestinal motility stimulant	Suppository	Janssen (Belgium)
Hydrocortisone	Dexocort	Mouth wash against aphthae, gingivitis, etc.	Solution	Actavis (Iceland)
Indomethacin	Indocid	Antiinflammatory agents	Eye drop	Chauvin (France)
Itraconazole	Sporanox	Esophageal candidiasis	Oral solution	Janssen (Belgium)
Mitomycin	Mitozytrex MitoExtra	Chemotherapeutic agent	Intravenous solution	SuperGen (USA) Novartis (Switzerland)
randomly methylated-b-cyclodextrin				
17- β -Oestradiol	Aerodiol	Postmenopausal hormone replacement therapy	Nasal spray	Servier (France)
Cloramphenicol	Clorocil	Antibiotic agent	Eye drops	Oftalder (Portugal)
sulphobutylether-b-cyclodextrin				
Voriconazole	Vfend	Antimycotic	Intravenous solution	Pfizer (USA)
Ziprasodine maleate	Geodon, Zeldox	Antischizophrenic	Intramuscular injection	Pfizer (USA)

CYCLODEXTRINS IN CONTROLLED DRUG DELIVERY SYSTEMS

From the viewpoint of the optimised pharmacotherapy, drug release should be controlled in accordance with the therapeutic purpose and the pharmacological properties of active substance. There has been growing interest in developing rate or time controlled oral preparations with the aim to achieve the zero-order or pH-independent drug release. This may provide a constant blood concentration of the drug for a prolonged period of time. Controlled release formulations offer the advantages such as reducing the dosing frequency, prolonging the drug efficiency and avoiding the toxicity associated with the administration of a plain tablet. To design such advanced dosage form, a suitable carrier materials are used to overcome the undesirable properties of drug molecules. Cyclodextrins are potential candidates for such role [24].

Hydrophobic cyclodextrins, such as alkylated and acylated derivatives are useful as slow release carriers for water-soluble drugs. Diethyl- and triethyl- β -cyclodextrin were the first slow release carriers used in conjunction with diltiazem and isosorbide dinitrate to obtain prolonged drug release in dogs. The peracylated cyclodextrins with medium alkyl chain lengths (C_4 - C_6) are particularly useful as novel hydrophobic carriers, because of their multifunctional characteristics, such as bioadhesive properties and ability to form films. Therefore, they can be used in oral and transdermal formulation of the drugs with short biological half-life [25].

Polymeric drug delivery systems, such as tablets, microparticles and hydrogels are often used to obtain the controlled drug release. The drug release from those formulations may be controlled by the swelling of the polymer matrix or by its erosion. The incorporation of hydrophilic cyclodextrins into polymeric drug delivery systems can modify the drug release pattern by increasing the drug solubility or diffusivity across the swollen polymer layer. Since hydrophilic cyclodextrins are readily soluble in water, they may act as channelling or wicking agents, improving hydration of the polymer matrix or promoting its erosion. This would allow the complete release of the drugs with low water solubility. Controlled release formulations of many drugs, such as carbamazepine, nicardipine, piroxicam, etc were successfully formulated using different polymers and hydroxypropyl-b- or sulphobutylether- β -cyclodextrin [26-29].

Osmotic pumps are the oral drug delivery systems in which the drug release is controlled by osmotic pressure. They are consisted of the drug reservoir, which can be either a solution or a solid formulation mixed with an osmotic agent enclosed with a semipermeable membrane. After oral application, the gastrointestinal fluid penetrates through the semipermeable membrane and dissolves the drug in the reservoir, as well as the osmotic agent. Under the created osmotic pressure, the drug is released continuously at a controlled rate through a vent pre-drilled by a laser beam. This kind of formulation may provide the zero-order drug release through a period of time up to 12 hours.

Sulphobutylether- β -cyclodextrin may be useful in formulation of the oral pumps, since it is a multifunctional agent. This cyclodextrin derivate possesses osmotic activity and it is an excellent drug solubilizer, providing the controlled and complete release of the drugs with low water solubility. The use of sulphobutylether- β -cyclodextrin in formulation of osmotic pump tablet for chlorpromazine gained prolonged and pH-independent drug release *in vivo*, without compromising the oral bioavailability of the drug [24,30].

Cyclodextrins may be used to obtain colon-specific drug delivery. Colon drug delivery systems are designed to permit targeted drug release in the terminal ileum and proximal colon. The objective of delivering drugs to these parts of the gastrointestinal tract is in providing of local delivery for treatment of colonic diseases, such as ulcerative colitis, colon cancer or constipation. Also, the drug absorbed via the colon will bypass the hepatic first pass metabolism. Further advantage of the drug delivery to colon may be obvious from the viewpoint of circadian biorhythm. The drug passage to the colon will occur approximately 8 hours after oral application. Therefore, colon delivery can provide a nocturnal release of drugs for diseases that are characterised by nighttime or early morning onset, such as asthma, hypertension, cardiac arrhythmias, etc. The drug/cyclodextrin inclusion complexes are not suitable for colon delivery due to their high solubility. The formation of drug/cyclodextrin conjugate, in which the drug is linked by ester bond to one of hydroxyl groups of the cyclodextrin molecule via a suitable spacer, would allow colon specific drug delivery. The cyclodextrin molecule produces steric hindrance to hydrolysis of the ester bond in upper part of the gastrointestinal tract thus prohibiting the drug absorption. The drug-cyclodextrin conjugates are first subjected to the cyclodextrin ring opening by bacterial enzymes in colon, followed by ester linkage hydrolysis and subsequent drug absorption in colon [25]. The formation of biphenylacetic acid conjugates with α - and γ -cyclodextrin provided colon specific drug delivery in rats, with 4 to 5 times higher oral drug bioavailability compared to the drug alone [31]. Ketoprofen/ α -cyclodextrin conjugate was demonstrated to be delayed-release type prodrug for colon-specific delivery. The combination of cyclodextrin conjugate with a ketoprofen/hydroxypropyl- β -cyclodextrin inclusion complex as a fast-release fraction yielded the repeated-release preparation. The oral application of this preparation showed strong anti-inflammatory effect on carrageenan-induced acute oedema in rat paw model during 12 hours after application. In the same time, the onset of drug action upon administration of this formulation was very fast [32].

OTHER POSSIBLE APPLICATIONS OF CYCLODEXTRINS IN PHARMACY

Advances in biotechnology have allowed the large-scale production of therapeutically important peptides offering the possibility of treating some until now untreatable diseases. But the therapeutical use of these compounds was hampered due to chemical

and enzymatical instability of the peptides, poor absorption through biological membranes, rapid plasma clearance, peculiar dose-response curves and immunogenicity. Different approaches have been used to solve this problem and cyclodextrin complexation seems to be an attractive solution.

The interaction between cyclodextrins and peptides or proteins is restricted since their molecules are too hydrophobic and bulky to be wholly included in the cyclodextrin cavity. Also, the topological constraints of the peptide backbone may reduce the inclusion complex formation. The interaction between polypeptides and proteins could be only local, including the inclusion complex formation between accessible hydrophobic residues of amino acids such as L-tryptophan, L-tyrosine and tertbutyl-D-serine. Therefore, cyclodextrins have been used to solubilize and stabilize various biomedically important peptides and proteins including growth hormone, interleukine-2, tumor necrosis factor, albumin, γ -globulin, etc. [33,34].

The property of insulin to form reversible and irreversible aggregates in solution leads to complication in the development of long-term insulin therapeutic systems. This limits the rate of insulin subcutaneous absorption, leading to the inadequate regulation of the blood glucose level after a meal. These problems are further complicated by the tendency of insulin to adsorb to the surface of a container. Some hydrophilic cyclodextrins, including maltosyl- and hydroxypropyl- β -cyclodextrin, significantly inhibit the absorption of insulin to hydrophobic surfaces of containers and its aggregation in neutral solution. Probably, cyclodextrins caused the perturbation of the intermolecular hydrophobic contacts between aromatic side chains across the monomer-monomer interfaces, leading to the inhibition of peptide self-association [35].

The cyclodextrins may have significant role in production of pharmaceutically important proteins. The particular problem in production of proteins by genetically engineered cells stems from the fact that proteins are overproduced in the form of cytoplasmic aggregates or inclusion bodies, in which proteins are misfolded and therefore functionally inactive. Weak interactions of cyclodextrins with unfolded proteins may enhance the solubility of denatured proteins by masking the exposed hydrophobic residues, thereby assisting the re-folding of the proteins. In this way cyclodextrins may act as artificial chaperones in the protein folding process [36].

As it was mentioned earlier, some hydrophilic cyclodextrin derivatives such as α - and randomly methylated- β -cyclodextrin are demonstrated to be potential permeation enhancers of the polypeptides across the biomembranes allowing systemic delivery of peptide and protein drugs via various mucosal routes (nasal, rectal, etc.). When compared with other permeation enhancers, they have rather mild and reversible effect on the mucosa. The effectiveness of cyclodextrins as permeation enhancers may be further upgraded by simultaneous application of other permeation enhancers or polymers such as chitosan [37].

The high external hydrophilicity of natural cyclodextrin, as well as their hydrophilic derivatives, reduces the possibility of intimate contact with biological membranes. For this reasons, several amphiphilic cyclodextrin derivatives has been synthesized. Amphiphilic cyclodextrins were obtained by grafting aliphatic chains of different length (C2-C18) by an ester bond on the primary hydroxyl ("medusa like cyclodextrins") or secondary hydroxyl groups of cyclodextrin molecule ("skirt shaped cyclodextrins"). Due to ester bond, this cyclodextrin derivatives are susceptible to being biodegradable. One of the interesting characteristics of amphiphilic cyclodextrins is their ability to spontaneously form nanospheres and nanocapsules without the presence of a surfactant (38). The nanoparticles based on amphiphilic cyclodextrin can be prepared directly from pre-formed drug/amphiphilic cyclodextrin inclusion complex using nanoprecipitation technique. The obtained nanoparticles have high drug loading capacity and reduced burst effect during the drug release [39]. This may be explained in terms of particular nanoparticles architecture made of organised cyclodextrin molecules that offers the opportunity to associate drug molecules at different levels within the nanostructure, i.e. surface adsorption, matrix entrapment or interaction with the cyclodextrin cavity. The size range of cyclodextrin-based nanoparticles (90-250 nm) is compatible with an intravenous administration and they can be sterilised by gamma irradiation [38, 40]. This sterilisation technique has insignificant influence on particle size, drug loading and drug release properties. According to the studies on human blood samples and L929 mouse fibroblast cells, amphiphilic cyclodextrins were reported to be non-hemolytic and non-cytotoxic [41, 42]. Recent *in vivo* studies have confirmed those results, since no particular signs of toxicity were observed after intravenous administration of amphiphilic cyclodextrins to rats [43]. This all indicates that cyclodextrin based nanoparticles may become important drug delivery system for drug targeting.

Another interesting field where cyclodextrins may be applied is in the gene delivery. The lack of safe and efficient gene-delivery methods is a limiting obstacle to human gene therapy. In recent years, a variety of effective polymers have been designed especially for non-viral gene delivery [44]. Among them, numerous cyclodextrin-containing polymers gained notable interest [45]. A common property of all cyclodextrin-containing polymers for nucleic-acid delivery is that they are polycations. One of important feature that is observed with cyclodextrin-contained polycations is their low *in vitro* and *in vivo* toxicity compared to other polycations without cyclodextrin molecule in their structure, such as poly-L-lysine and polyethylenimine [1,45]. Cationic polymers are able to deliver nucleic acid into cells by self-assembling with the anionic DNA via electrostatic interactions to subsequently form positive charged particles with size up to 500 nm, that are taken up by cells.

The important feature unique to the cyclodextrin-containing cationic polymers is that they can be modified by compounds capable of forming inclusion complexes. This may be achieved using polyethylene glycols compounds terminated with adamantine, that form inclusion complexes with cyclodextrins on the particle surface. This method of surface modification does not involve the portions of polycations that bind to the DNA, therefore the disruption of particle is avoided. Obtained surface modification of cyclodextrin-containing cationic particles provided sterical stabilisation and reduction of the elimination rate from the blood stream by phagocytes. This approach has been extended to cyclodextrin-grafted polyethylenedimines and shown to produce delivery vehicles that can target tumour and liver tissue in mice with either plasmids or oligonucleotides [46,47]. It is likely that gene delivery systems based on cyclodextrin-containing polycations might become an important tool for human gene therapy.

CONCLUSION

Cyclodextrins are known more than 100 years. This period of time was necessary for the evolution of cyclodextrins from interesting chemical oddities to enabling pharmaceutical excipient. Due to their biocompatibility and multifunctionality, cyclodextrins may be used to alleviate undesirable characteristics of the drugs. In the same time, cyclodextrin based technology is continuously developing, therefore cyclodextrins still may be regarded as novel excipients of unexplored potential.

References

- [1] Davis ME, Brewster ME. Cyclodextrin based pharmaceuticals: past present and future. *Nat Rev Drug Discovery* 2004;3:1023-1035.
- [2] Loftsson T, Jarho P, Mässon M, Järvinen., Cyclodextrins in drug delivery. *Expert Opin Drug Deliv* 2005;2:1-17.
- [3] Frömming KH, Szejtli J. Cyclodextrins in pharmacy. Dortrecht: Kluwer Academic Publishers; 1994, pp. 1-81.
- [4] Larsen KL. Large cyclodextrins. *J Incl Phen Macrocyclic Chem* 2002; 43:1-13.
- [5] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Del Rev* 2007;59:645-666.
- [6] Irie T, Uekama K. Pharmaceutical applications of cyclodextrins: III. Toxicological issues and safety evaluation. *J Pharm Sci* 1997;86:147-162.
- [7] Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical application. *Int J Pharm* 2007;329:1-11.
- [8] Szejtli J. Cyclodextrin complexed generic drugs are generally not bioequivalent with the reference products: Therefore the increase in number of marketed drug/cyclodextrin formulations is so slow. *J Incl Phen Macrocyclic Chem* 2005;52:1-11.

- [9] *Rajpurkar A, Dhabuwala CB.* Comparison of satisfaction rates and erectile function in patients treated with sildenafil, intracavernous prostaglandin E₁ and penile implant surgery for erectile dysfunction in urology practice. *J Urol* 2003;170:159-163.
- [10] *Szejtli J, Szente L.* Elimination of bitter, disgusting tastes of drugs and food by cyclodextrins. *Eur J Pharm Biopharm* 2005;61:115-125.
- [11] *Lofstsson T, Hereinsdóttir D, Másson M.* Evaluation of cyclodextrin solubilization of drugs. *Int J Pharm* 2005;302:18-28.
- [12] *Lofstsson T, Järvinen T.* Cyclodextrins in ophthalmic drug delivery. *Adv Drug Del Rev* 1999;36:59-79.
- [13] *Lofstsson T, Stefánsson E.* Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye. *Acta Ophth Scand* 2002;80:144-150.
- [14] *Lofstsson T, Másson M.* The effects of water-soluble polymers on cyclodextrins and cyclodextrin solubilization of drugs. *J Drug Del Sci Tech* 2004;14:35-43.
- [15] *Merkus FWHM, Verhoef JC, Martin E, Romeijn SG, van der Kuy PHM, Hermens, WAJJ, Schipper NGM.* Cyclodextrins in nasal drug delivery. *Adv Drug Del Rev* 1999;36:41-57.
- [16] *Illum LB.* Nasal drug delivery – possibilities, problems and solutions. *J Controlled Release* 2003;87:187-198.
- [17] *Studd J, Pornel B, Marton I, Bringer J, Varin C, Tsouderos Y, Christiansen C.* Efficacy and acceptability of intranasal 17 β -oestradiol for menopausal symptoms: randomised dose-response study. *Lancet* 1999;353:1574-1578.
- [18] *Pelisser C, de Kervasdoue A, Choung VT, Maugis EL, de Mouillac FV, Breil MH, Moniot G, Zeitoun-Lepvirier G, Robin M Rime B.* Clinical evaluation, dose-finding and acceptability of AERODIOL®, the pulsed estrogen therapy for treatment of climacteric symptoms. *Maturitas* 2001;37:181-189.
- [19] *van der Kuy PHM, Lohman JJHM, Hooymans PM, Ter Berg JWM, Merkus FWHM.* Bioavailability of intranasal formulations of dihydroergotamine. *Eur J Clin Pharmacol* 1999;55:677-680.
- [20] *Asai K, Morishita M, Katsuta H, Hosoda S, Shinomiya K, Noro M, Nagai T, Takayama K.* The effect of water-soluble cyclodextrins on the histological integrity of the rats nasal mucosa. *Int J Pharm* 2002;246:25-35.
- [21] *Agu RU, Jorissen M, Willems T, den Mooter GV, Kinget R, Verbeke N, Augustijns P.* Safety assessment of selected cyclodextrins – effect on ciliary activity using a human cell suspension culture model exhibiting in vitro ciliogenesis. *Int J Pharm* 2000;193:219-226.
- [22] *Lofstsson T, Brewster ME, Másson M.* Role of cyclodextrins in improving oral drug delivery. *Am J Drug Deliv* 2004;2:261-275.
- [23] *Lee CR, Balfour JA.* Piroxicam- β -cyclodextrin. A review of its pharmacodynamic and pharmacokinetic properties and therapeutical potential in rheumatic diseases and pain states. *Drugs* 1994;48:907-929.

- [24] Hirayama F, Uekama K. Cyclodextrin-based controlled drug release systems. *Adv Drug Del Rev* 1999;36:125-141.
- [25] Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. *Chem Rev* 1998; 98:2045-2076.
- [26] Bibby DC, Davies NM, Tucker IG. Mechanism by which cyclodextrins modify drug release from polymeric drug delivery systems. *Int J Pharm* 2000;197:1-11.
- [27] Quaglia F, Varricchio G, Miro A, La Rotonda MI, Larobina D, Mensitieri G. Modulation of drug release from hydrogels by using cyclodextrins: the case of nicardipine/b-cyclodextrin system in crosslinked polyethyleneglycol. *J Controlled Release* 2001; 71:329-337.
- [28] Jug M, Bećirević-Laćan M. Influence of hydroxypropyl-b-cyclodextrin complexation on piroxicam release from buccadhesive tablets. *Eur J Pharm Sci*, 2004 21: 251-260.
- [29] Smith JS, MacRue RJ, Snowden MJ. Effect of SBE-b-cyclodextrin complexation on carbamazepine release from sustained release beads. *Eur J Pharm Biopharm* 2005; 60:73-80.
- [30] Okimoto K, Ohike A, Ibuki R, Aoki O, Ohnishi N, Irie T, Uekama K, Rajewski RA, Stella VJ. Design and evaluation of an osmotic pump tablet for chlorpromazine using (SBE)₇- β -CD. *Pharm Res* 199; 16:549-554.
- [31] Miniemi K, Hirayama F, Uekama K. Colon specific drug delivery based on a cyclodextrin prodrug: release behaviour of biphenylacetic acid from its cyclodextrin conjugates in rat intestinal tract after oral administration. *J Pharm Sci* 1998; 87:715-720.
- [32] Kamada M, Hirayama F, Udo K, Yano H, Arima H, Uekama K. Cyclodextrin conjugate-based controlled release system: repeated- and prolonged- releases of ketoprofen after oral administration in rats. *J Controlled Release* 2002;82:407-416.
- [33] Irie T, Uekama K. Cyclodextrins in peptide and protein delivery. *Adv Drug Del Rev* 1999;36:101-123.
- [34] Aachmann FL, Otzen DE, Larsen KL, Wimmer R. Structural background of cyclodextrin-protein interactions. *Protein Eng* 2003;16:905-912.
- [35] Katkam M, Banga AK. Aggregation of insulin and its prevention by carbohydrate excipients. *J Pharm Sci Tech* 1995;49:160-165.
- [36] Karupiah N, Ssharima A. Cyclodextrins as protein folding aids. *Biochem Biophys Res Commun* 1995;211:60-66.
- [37] Junginger HE, Verhoef JC. Macromolecules as safe penetration enhancers for hydrophilic drugs – a fiction? *PSTT* 1998;9:371-376.
- [38] Duchene D, Ponchel G, Wouessidjewe D. Cyclodextrins in targeting, application to nanoparticles. *Adv Drug Del Rev* 1999;36:29-40.
- [39] Memisoglu E, Bochot A, Ozalp M, Sen M, Duchene D, Hincal AA. Direct formation of nanospheres from amphiphilic beta-cyclodextrin inclusion complexes. *Pharm Res* 2003;20:117-125.

- [40] Memisoglu-Bilensoy E, Atila Hincal A. Sterile, injectable cyclodextrin nanoparticles: Effects of gamma irradiation and autoclaving. *Int J Pharm* 2006;311:215-222.
- [41] Memisoglu-Bilensoy E, Dogan AL, Hinchal AA. Cytotoxic evaluation of injectable cyclodextrin nanoparticles. *J Pharm Pharmacol* 2006;58:585-589.
- [42] Memisoglu-Bilensoy E, Vural I, Bochot A, Renoir JM, Duchene D, Hincal AA. Tamoxifen citrate loaded amphiphilic b-cyclodextrin nanoparticles: In vitro characterisation and cytotoxicity. *J Controlled Release* 2005;104:489-496.
- [43] Gèze A, Tieu Chau L, Choisnard L, Mathieu JP, Marti-Batlle D, Riou L, Putaux JL, Wouessidjewe D. Biodistribution of intravenously administered amphiphilic α -cyclodextrin nanospheres. *Int J Pharm* 2007; doi:10.1016/j.ijpharm.2007.06.050.
- [44] Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nat Rev Drug Discovery* 2005;4:581-593.
- [45] Davis ME, Belloq NC. Cyclodextrin-containing polymers for gene delivery. *J Incl Phen Mol Recognit Chem* 2002;44:17-22.
- [46] Belloq NC, Pun SH, Jensen GS, Davis ME. Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery. *Bioconjugate Chem* 2003; 14:1122-1132.
- [47] Pun SH, Belloq NC, Liu AJ, Jensen G, Machemer T, Quijano E, Schluep T, Wen SF, Engler H, Heidel J, Davis ME. Cyclodextrin-modified polyethylenimine polymers for gene delivery. *Bioconjugate Chem* 2004;15:831-840.

Sažetak

Ciklodekstrini u ljekovitim oblicima

Ciklodekstrini, ciklički oligosaharidi, predstavljaju skupinu farmaceutskih pomoćnih tvari koja je svojom multifunkcionalnošću privukla značajan interes. Temeljni razlog njihove popularnosti je u svojstvu ciklodekstrina da stvaranjem inkluzijskih kompleksa mijenjaju nepovoljne karakteristike lijekova, kao što je neugodan okus, niska topljivost u vodi i kemijska nestabilnost. Također, ciklodekstrini mogu povećati bioraspoloživost lijeka neovisno o putu primjene. Zahvaljujući biokompatibilnosti i netoksičnosti, ciklodekstrini se mogu koristiti u ljekovitim oblicima namijenjenim za različite putove primjene, uključujući oralni, okularni, nazalni te parenteralni put. Cilj ovog članka je istaknuti važnost i funkcionalnost ciklodekstrina u različitim ljekovitim pripravcima te naznačiti smjerove daljnjeg razvoja.

Glavne riječi: ciklodekstrin; inkluzijski kompleks; primjena lijeka; topljivost u vodi; kemijska stabilnost; bioraspoloživost.

Symposium

55 YEARS FROM ORGANIZING THE BEGINNING
OF ALLERGOLOGY IN CROATIA:
FUTURE WE BUILT ON THE PAST

SALOMON STRICKER (1834-1898): A PIONEER MICRO-BIOLOGIST

Karl Holubar

Institute for the History of Medicine, Medical University of Vienna

Summary

The paper presents basic biographical data on the Austrian pioneer of experimental medicine Salomon Stricker (1834-1898), and emphasizes the link of his experimental work to important research projects of this time period. His influence on Carl Heitzmann and Carl Koller was pointed out. The work of both was connected directly with Stricker's experimental designs, far less known in published literature. Demonstrating *ad oculos* the world of capillaries, diapedesis of blood cells, cell division *in vivo* by Stricker, paved the way for investigations in immunology and allergology.

Keywords: Salomon Stricker (1834-1898); development of experimental medicine; Austria/Vienna, 19th century.

Erna Lesky (1911-1986), Vienna's *grande dame* of the field started the part on Stricker in her classic on medical history in the city (1, p.544 ff.), saying that "it did not cause little attention that the dominant personality in pathology, Carl von Rokitansky (1804-1878), who previously claimed that pathology must be the basis of all action in medicine, now favored the foundation of a department for experimental pathology. Salomon Stricker (1834-1898) was chosen, coming from Ernst Wilhelm von Brücke's (1819-1892) laboratory. He had a sound laboratory experience already as a student (1855-1859) in the latter's laboratory, worked also with Carl Ludwig (then at our own institution in the school of military medicine). Graduating in 1858 he underwent clinical training in the various departments of the Vienna General (i.e. University) Hospital in internal medicine, surgery, dermatology, syphilology ophthalmology, and already after four years became docent.

Correspondence to: Professor Karl Holubar MD FRCP GSE, Professor of Dermatology and of the History of Medicine, The Max and Margareta Wolf Library for the History of Dermatology, Institute for the History of Medicine, Medical University of Vienna, Waehringer Strasse 25, A-1090 Vienna, Austria, email:karl.holubar@meduniwien.ac.at



Fig. 1. Salomon Stricker 1834-1898 (Courtesy and copyright by Prof. P. Usenský, Vice-Rector, University of Bratislava, Slovakia).

In 1868 the institute of “Allgemeine und experimentelle Pathologie” was founded, Stricker was appointed Professor extraordinarius, to reach the highest and most coveted rank of Professor ordinarius only after five more years in 1873. A phenomenal career [2,3] Just for comparison: Abraham Romeo Seligmann (1808-1892), first professor of history of medicine in 1848, it took 21 years; Carl Ludwig Sigmund von Ilanor (1810-1883), first professor of syphilis 1849 and Ferdinand Hebra (1816-1880), first professor of skin diseases also 1849, it took both twenty years, Moriz Kaposi (1837-1902) and Isidor Neumann (1832-1904), their successors it took 12 years, to bridge this gap, which by itself had only a character of higher prestige and a voting right in the faculty.

Stricker was of humble origin, born in the then Hungarian City of Vág-Ujhely/Waag Neustadt, today Novo Mesto nad Vahóm, Slovakia. He was Jewish; first attending the Catholic German gymnasium in Pressburg/Pozsony, today Bratislava, capital of Slovakia, thereafter the Protestant gymnasium in Budapest. Medical School he made in Vienna and there he stayed ever after. In the perspective of what is a chronicler, what a historian and what an experimenter, Stricker definitively fell into the latter category but with all the knowledge the former two categories would require. He must have had two outstanding qualities: (i) an extraordinary IQ plus a strong drive for making a career – well in line with historical scholarly tradition of Jewish education- and (ii) an equally remarkable dexterity in manual activities. His projection devices he all designed and built by himself, according to his followers, and thereby was able to demonstrate

experiments on the walls of his lecture theaters in a final magnification of 10.000-20.000. Some achievement at the time, indeed. As a parameter may be taken, that his lectures were all chock-full of attendees, students and contemporaries alike. As any lecturer or professor well knows, both these categories of listeners are the most difficult to convince, to impress, to teach. Let me just mention from the vantage point of the dermatologist, who were his pupils in this particular field making him, historically, the main path into dermato-pathology as finally designed by Paul Gerson Unna (1850-1929). Albert Reder-Schellmann, later ordinarius for dermatology at the military medical academy in Vienna (1826-1904), Isidor Neumann (1832-1904), ordinarius for syphilis and dermatology 1881-1904; Filipp Josef Pick (1834-1910), for the same disciplines professor in Prague 1873-1910, Heinrich Auspitz (1835-1886), professor and head of a non-university department of syphilology in the Vienna General Hospital, Moriz Kaposi (1837-1902), successor to Ferdinand Hebra in the prestigious chair of Dermatology in Vienna (1881-1902), Eduard Lang (1841-1916), first professor in the same field in Innsbruck (1873-1886), the follower to Auspitz in Vienna, nad the above mentioned Paul Gerson Unna. He is the editor of the first encyclopedic volume of dermatopathology in 1894 (German) and 1896 (English) even if Isidor Neumann's treatise of 1869 and after (five editions) give many details of the dermatopathology, e.g. of lupus erythematoses (sic!), or of senile skin changes well before Unna. The years of birth of the above gentlemen may illustrate how well Stricker's talents were perceived amongst his contemporaries otherwise they would not have attended his presentations [2].

Stricker is described by his followers and pupils as a workaholic. His motto was "*docendo discimus*", teaching was the core of his scientific activities. The "*Handbuch der Gewebelehre*" and the series of articles in the Vienna Weekly on experimental pathology amply offer evidence for his theories and perspectives. This notwithstanding, he got into an argument with Julius Cohnheim, his erstwhile friend, on the theories of inflammation. He did not enter bacteriology coming up first with Armauer Hansen's description of the leprosy bacillus nor with Koch's detection of the mycobacterium tuberculosis. Well worth to mention however are three other facts:

- (i) Carl Heitzmann (1836-1896) the famous, Croatian born (Regnum Hungariae and Austrian Empire of then, just like Stricker) [3], dermatologist and pathologist and physician painter, founding member of the American Dermatological Association (ADA, the world's oldest and most prestigious national dermatological society), in 1876, after his emigration to the USA, was one of his most avid followers and pupils [4].
- (ii) The demonstration of the diapedesis of erythrocytes in vivo as much as the contractibility of capillaries, which he was able to demonstrate to Cohnheim.

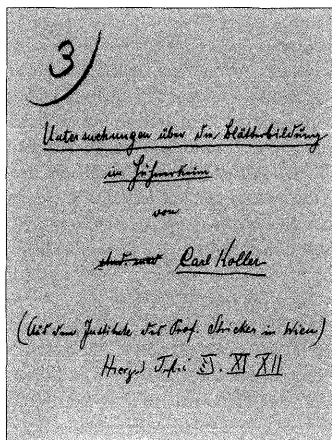


Fig. 2. frontispiece of a manuscript draft by Carl Koller from the early 1880s, obviously just around Koller's graduation (1882), because it reads "stud. med", crossed out thereafter when he must have become M. D. already. Copyright by Madame Hortense Koller-Becker, 1902-2002.

- (iii) Carl Koller (1857-1944), who introduced cocaine into ophthalmology, working on that drug together with Sigmund Freud (1856-1939) and Sigmund Lustgarten (1857-1911), was stimulated to perform such experiments under Stricker's original domain. A factor widely overlooked in the literature (Fig 2).

Koller's first report on cocaine for ophthalmo-anaesthesia appeared in 1884 [5,6]. Both, Koller and Lustgarten left Austria. In 1884, Koller entered into a duel, won, but both opponents had to quit the Imperial Army, and Koller left the following year. Lustgarten, his assistant in the duel, followed after a controversy with the administration of the Vienna General Hospital in 1887. He settled in New York City, in his obituaries described as a "docteur à la mode" and "Schoengeist", but he retained emotional ties to his old country. In 1910 he made a will favoring the Medical Faculty of Vienna University and the Mount Sinai Hospital in New York City, to benefit talented students of any denomination. He died in 1911; the will was enacted after the death of his wife in 1941 but, due to WW II was effectuated only in the mid-fifties. The author of these lines was the first (or one of the first) Lustgarten stipendiates, - warranting perennial gratitude on the part of the writer.

As with many devoted, enthusiastic investigators, scientists, private life lags behind Stricker married when he was beyond fifty, his bride was twenty. It must have been love, which falls upon man like a golden cage from above. After eight years of marriage



Fig 3. Madame Ann M., Sydney.

he died – and he left her a fortune. She could easily have smiled to another guy, but steadfastly remained a widow for 37 years, lagging only behind Martha Hebra, Kaposi´s spouse, who outlived her husband 44 years, Margarethe Langerhans, 45 years, and Zita Hapsburg, 67 years. Regrettably, Aloisia Stricker´s fortune was invested in War Loans in WW I and eventually she died in dire poverty on Christmas Day 1935 in Vienna.

The author of this short article is in touch with many of the old Viennese medical families, many of them Jewish: Sigmund, Hebra, Kaposi, Urbach, Koller, Stricker, et al. Stricker had no children, but his brother Abraham had and the progeny is spread over the globe, Australia, USA and beyond. With her kind permission, the portrait photograph of Stricker´s great-grand niece, Madame Ann M. of Sydney, whom the author knows personally, a reproduction of her image appears here as Fig. 3.

In conclusion it can be said that Stricker at the microscope level as much as Hebra on the clinical level (dermatitis artefacta; experimental scabies) were pioneers in experimental medicine. *Allergology* of today, by the mere token of immunological mechanisms operative in the antigen-antibody arena, is the playground of modern scientists, exemplified in the new “biologicals” which were beyond anybody´s dreams just a quarter of a century ago. Stricker paved the way by demonstrating *ad oculos* the world of capillaries, diapedesis of cells, cell division *in vivo* and more.

References

- [1] Lesky E. Die Wiener medizinische Schule im 19. Jahrhundert. Wien: Böhlau; 1965.
- [2] Holubar K. Salomon Stricker (1843-1898). Pioneer experimental pathologist. Am. J. Dermatopathol 1987;9:149-150.

- [3] *Crissey JT Parish LC, Holubar K. Historical Atlas of Dermatology and Dermatologists. London: Parthenon; 2002.*
- [4] *Fatović-Ferenčić S, Plewig G, Holubar K. Skin in Water Colours. Berlin-Oxford: Blackwell; 2003.*
- [5] *Tragl K. Chronik der Wiener Krankenanstalten. Wien: Böhlau; 2007.*
- [6] *Amm M. "Coca-Koller" und seine Freunde. Zum 140. Geburtstag des jüdisch-wienerischen Trios: Carl Koller (1857-1944), Sigmund Lustgarten (1857-1911) und Sigmund Freud (1856-1939) Wien. Klin.Wschr. 1997;109:170-175.*
- [7] *Holubar K Sigmund Lustgarten (1857-1911). A forgotten dermatologist, researcher, philanthropist and gentleman. Dermatology: Practical and Conceptual 2006;11:1-11.*

Acknowledgments

Madame Ann M., Sidney and Prof. Dr. P. Osuský, Vice – Rector of the Comenius University of Bratislava, Slovakia

Sažetak

Salomon Stricker (1834.-1898.): Pionir mikrobiologije

U radu se iznose osnovni biografski podaci o austrijskom pioniru eksperimentalne medicine Salomonu Strickeru (1834.-1898.) te se podvlači poveznica njegova eksperimentalnog rada s važnim istraživačkim projektima tog vremena. Osvjetljen je utjecaj Strickera na Carla Heitzmanna (1836.-1896.) te na Carla Kollera (1857.-1944.) koji je uveo kokain u oftalmologiju. Strickerovom eksperimentalnom utjecaju pripisuje se Kollerov istraživački interes, što je do sada bilo nepoznato u objavljenoj literaturi. Stricker je istražujući ulogu kapilara staničnu diapedezu i diobu utro put kasnim istraživanjima na području fiziologije, imunologije i alergologije.

Ključne riječi: Salomon Stricker (1834.-1898.); razvoj eksperimentalne medicine; Austrija/Beč, 19. stoljeće

BEGINNINGS AND DEVELOPMENT OF ALLERGOLOGY IN CROATIA FROM 1870 TO 1970

Stella Fatović-Ferenčić

Department for the History and Philosophy of Sciences, Division for the History of
Medicine, Croatian Academy of Arts and Sciences, Zagreb

Summary

Development of allergy as an immunologic and experimental phenomenon in Croatia went similar as in surrounding neighboring countries. In the present article, I describe the most important events and discoveries that led to the understanding the origin of allergy in the 1870-1970 period. The crucial moments in the development of allergology in Croatia were first linked to the production of vaccines and foundation of the Institute of Immunology and its forerunners in the first half of the 20th century. Establishment of these institutions represented a strong institutional vertical that has played a key role in the development of allergology and immunology in Croatia. The analysis of articles published in *Liječnički vjesnik* in the 1877-1977 period showed the extent to which allergology was represented and the roles of the most important figures in the field of allergology, such as internist Ivan Hugo Botteri (1876-1963), pediatrician Ernest Mayerhofer (1877-1957), and dermatovenereologist Franjo Kogoj (1894-1983), who paved the way to the scientific research and roused the interest of physicians in this medical area. However, *Liječnički vjesnik* is not representative source for the development of allergology for the whole analyzed time period due to the fact that since 1960-ies large part of Croatian immunologists and allergologists started to publish the best part of their results within various international publications. Gathering of different medical specialists who were interested in allergology resulted in the establishment of Allergology Section of the Croatian Medical Association in 1952. The Section played an important role in the education of a wider academic community through symposia, congresses, and seminars. The analyzed records have shown that the institutional and physicians' activities in the first half of the 20th century laid a firm foundation to modern research, subspecialties, and allergologic centers and units within different medical institutions across Croatia.

Keywords: *history of medicine – Croatia; development of allergology – Croatia; Liječnički vjesnik – allergy; 19th century; 20th century.*

Correspondence to: Stella Fatović-Ferenčić, Department for the History and Philosophy of Sciences, Division for the History of Medicine, Croatian Academy of Arts and Sciences, Zagreb, Gundulićeva

The mechanism and appearance of allergic reactions have not changed over time. The biological constancy of this phenomenon is evident from many medical texts of ancient writers. There are records of asthmatic attacks and cases of hypersensitivity from Medieval times as well as later centuries. However, the key events in the development of allergology as a modern medical discipline include a series of discoveries in the 19th and 20th centuries. If we go back to the early 1800's, we find Bostock's description of hay fever from 1819 and Magendie's observations on the occasional sudden death of rabbits injected with egg albumin from 1839. Once it was shown that animals could be immunized by immune serum to the toxins produced by the diphtheria and tetanus bacilli, the attention shifted to humoral immunity. Opposing the view that resistance to the bacteria resides in the serum, a Russian pathologist Ilya Ilych Metchnikoff offered a counter theory of phagocytes, and the dispute became the scientific expression of Franco-German and Russo-Japanese rivalry [1]. At the beginning of the 20th century, the significance of early discoveries was brought into greater focus. To describe increased sensitivity to and the absence of protection against the effects of toxin in immunized animals, Richet and Portier introduced the term *anaphylaxis*. Their investigations were later continued and extended by numerous other investigators across Europe, such as Maurice Arthus, who demonstrated the possibility of inducing local anaphylaxis in rabbits, and Bela Schick and Clemens von Pirquet, two pediatricians from Vienna who recognized that the clinical features of serum sickness differed from those of infectious diseases and suggested close links between human reactions to foreign sera and experimental anaphylaxis in animals, thus paving the way to the idea of immunological basis of anaphylaxis. Various immunological research findings were given greater clarity by von Pirquet, who, in 1906, offered a semantic framework for classification and better understanding of different phenomena within the domain of immunology [2].

The key events and discoveries in the field of immunology and allergology soon resounded in Croatia. In this article, the analysis of allergology development in our geographical area in the 1870-1970 period is focused on the beginnings and development of institutionalized vaccination against infectious diseases and the earliest work of Croatian physicians published in *Liječnički vjesnik* (Table 1). Special attention is paid to the role of the most important figures in the first half of the 20th century responsible for raising the interest of medical community in immunology and allergology and paving the way for scientific research in this area.

FROM SERUM THERAPY TO SERUM SICKNESS

In Croatia as well as in Europe, the beginnings of allergology were closely linked with public health actions related to the vaccination of population. The 19th century was a period when mass vaccination and systematic antitoxin treatment brought bacterial

disease surveillance, diagnosis, treatment, and prevention closer to the center of interest. In the 1870's in Croatia, there were intensified endeavors to vaccinate population with animal vaccines and produce adequate vaccines, which culminated in the establishment of the Institute for Production of Small Pox Vaccine upon the initiative by Izidor Schlick, a county physician and city coroner, in Bjelovar in 1890. The following year, the Law on Pox Vaccination was passed in the Kingdom of Croatia and Slavonia; the first article of the Law specified that small-pox vaccination was to be performed *only with animal vaccine*. In 1893, the Royal Institute for Production of Animal Small-pox Vaccine was founded in Zagreb, joining fifteen other similar institutes that had already been established across Austro-Hungarian Empire [3,4].

At the beginning of the 20th century, the term *allergy* did not exist and neither did the knowledge about the mechanisms of that disease, today classified under the disorders of allergic or immunologic origin. Even when Clemens von Pirquet coined the word *allergy* in 1906, this nosologic concept attracted only a limited number of researchers and only to the extent related to the pathogenesis and therapy of serum sickness. Since health care priorities at that time were high child mortality, especially from infectious diseases, it is understandable that hay fever seemed a comparatively peripheral problem. In Croatia, increasing efforts were being made with respect to mass vaccination and administration of antitoxin and serum against variola and rabies, and later on against dysentery, typhus, and cholera. These activities resulted in the foundation of the first Institute for Human Microbiology and Chemistry in Zagreb in 1907, primarily due to the persistency of Ljudevit Gutschy [3,4]. However, it soon turned out that serum could have not only unwanted, but also fatal consequences for human health, as Pirquet and Schick [5] explained in length in their article on serum sickness in 1905. Croatian physicians published their experience with serum administration as well. The first one who described the consequences of antidiphtheric serum administration was Oto Müller [6]. Here is what he wrote in his article from 1912:

A death due to anaphylaxis after the third injection of antidiphtheric serum
I had the following case. A 3-year-old child took ill with diphtheric croup in late December of 1910. It was the third attack of the disease; the first one occurred in December 1908, and the second one in March 1910. The first attack, according to the parents, was over 24 hours after the injection without any further consequences: the signs of the so-called serum sickness, *Serumkrankheit*, did not appear. How much antitoxin the child received, and how much serum, I could not learn. On the occasion of the second attack, I injected 3000 A. U. in 12 ccm of serum; in 30 hours, the serum was successful again and the child was free of disease, but 8 days later after the serum injection exanthema appeared again. The third time the disease occurred at the end of the previous year, as early as 9 months after the

second attack; the peril of anaphylaxis was that much greater as the child was be injected with horse serum for the third time within the interval of only 2 years. Still hoping that the child only had pseudocroup – diphtheritic layers in the pharynx could not be observed – I tried to alleviate the stenosis with inhalations, and hot baths. But all in vain: the stenosis advanced in only three hours so much that I decided with heavy heart, warning the parents about the peril, to try with the third injection. I gave the child 2000 A. U. in 4.5 ccm of serum. The next morning, the child's breathing improved, the stenosis was smaller, the voice stronger, the pulse and sensorium relatively favorable. But around noon, there was a sudden collapse without any clearer pre-symptoms. The child managed to come to life with help of camphor, caffeine, alcohol, and heart massage, but remained soporous until the death. The pulse was between 150-160, of poor quality, breathing indeed was not stenoctic anymore, but the child was nevertheless dyspnoic (40-50 breaths per minute); at 3 o'clock in the morning, 30 hours after the injection, the child passed away. We are to assume that death was caused by anaphylaxis, which developed due to reinjection of horse serum. If, namely, an animal parenterally, i.e. „outside of intestines“, receives albuminate of another species, e.g., serum, than after a particular length of time, the animal develops anaphylactic disposition. If the same serum is injected at that stage, even in a negligible amount which, like the first injection, could do absolutely no harm, then the animal, after the injection of the same serum, becomes very sick, and is often killed. According to the interpretation of Ehrlich's *Seitenkettentheorie*, after the first injection of any heterologic serum, specific so-called „antikörper“ are created in the organism in a significantly greater amount than what would be needed for neutralization of the antigens to the first injection. These „antikörper“ circulate in blood, sometimes for many years. With the injection of the same serum, the newly formed „antikörper“ couple with the antigen significantly faster and in greater extent than after the first injection. This biological process is always coupled with the decomposition of protein, a process considered to be similar to the decomposition to which the protein is subjected during digestion in the intestines, only that the decomposed protein, if it circulates in the blood, affects the organism like poison, and is therefore to be considered the cause of anaphylactic phenomenon. In our case, the alternative was as follows: either to observe calmly while the child suffocates from diphtheritic croup or to counteract diphtheria with antitoxic serum, ensuring for the amount of serum to be as small as possible to avoid the peril of anaphylaxis. As long as we do not have at our disposal a serum which originates from an animal other than horse, we cannot do anything differently in cases such as this one [6].

In those times, diphtheria was one of the greatest public health problems. The disease had a severe clinical picture, croup – mostly tonsillary – frequently occurred, and descending tracheal diphtheria was often accompanied by severe pseudodiphtheritic paralysis. The above case report written by the hand of a private practitioner, railway physician, and pediatrician in Zagreb, Oton Müller, indicates how grave a situation was for patients as well as physicians who just met with the fatal phenomenon of hypersensitivity. Serum sickness and other manifestations of allergies raised numerous questions and doubts, which became even more acute after Pirquet's attempt to provide a common semantic frame for these phenomena, so that they could be classified and investigated.

For Pirquet, the crucial question was the relationship between the immunity and hypersensitivity. He suggested the term *allergy* to designate a condition of changed organism reaction. He considered immunity and hypersensitivity mutually opposite, but closely related, manifestations of allergy and tried to prove that allergy was the result of the reaction of organism rather than the result of toxic effect of allergens. Thus he set the foundations to allergology [7].

Physicians in Croatia shared similar thoughts. Institutionalization of vaccine production and practical experience with vaccine administration in Croatia were fertile soil for the development of immunology, at the time a discipline in its formative stage, as illustrated in the article published by Ljudevit Gutschy in *Liječnički vjesnik* in 1908, intended to inform a wider readership on the role of immunology in the development of medicine [8]. On the other hand, the rapid evolution of biochemistry made studies in chemical properties of antibodies and nature of the antigen-antibody bond a rewarding research subject. Studies into specificity of immunological reactions brought immunology closer to the more advanced disciplines, such as chemistry. A series of articles by Fran Bubanović, published in 1913, are a good illustration of this statement. In these articles, the author describes the multiplicity of attitudes toward the area and role of immunochemistry, summing them up as follows [9]: *From this discussion it may be learned that studies in an interesting and important field of serotherapy and science of immunity have yielded several theories, in addition to abundant material, related to close observation of experimental data, the theories that are conveniently called immunochemistry. Quantitative examination of experimental material, first introduced by Paul Ehrlich, is the basis of immunochemistry. The diversity of the aforementioned theories, as well as contradictory experimental data, have its roots in the lack of knowledge about chemism and lability of substances, which are activated during immunochemical reactions. But in the end, we must admit that despite all the difficulties and complicated circumstances, we somewhat managed to take a glance at the quantitative situation in the field, so there is no doubt that, with advancement in all branches of life sciences coming into contact in this important field, the first steps will continue further and*

even deeper into area of serotherapy and science of immunity. Equally justified is the hope that the scientific work in this field will be crowned with practical results that can be successfully used in the field of therapy [9].

Considering all said above, we would expect to find an increasing number of case reports on hypersensitivity. However, there were only two from early decades of the previous century, published in *Liječnički vjesnik*. In addition to the aforementioned article by Oton Müller from 1923, written by Dr. Aleksandar Stangl, physician in Vukovar county entitled *An interesting case, serum anaphylaxis* [10].

FROM REVIEW ARTICLES TO SCIENTIFIC RESEARCH

Pirquet's neologism was not well received by his peers. In 1912, Charles Richet, who received the Nobel Prize in 1913 "in recognition of his work on anaphylaxis," suggested that the term was redundant. But, dismissals of von Pirquet's formulation proved premature. Certain reservations toward the use of the term *allergy* were evident in writings of Croatian medical writers. If we look at the chronological sequence of articles on allergies published on the pages of *Liječnički vjesnik* (Table 1), we can see that the first two review articles carry the title *Anaphylaxis* [11,12]. By listing individual case reports on hypersensitivity from the literature, Bogomil Kohout writes: *This complex of symptoms, which sometimes appeared after repeated injections of different sera, was called after Richet – anaphylaxis*. He further states that the absolute immunity for revaccination can never be obtained and that the reaction always appears. In the same article, he introduces the readership to the results of experiments on guinea pigs, which were performed by Arthus (Arthus' phenomenon), Rosenau, Anderson, Smith (Smith's phenomenon) and others, and writes about passive and active anaphylaxis, emphasizing that passive is shorter and lasts 13-15 days. Anaphylaxis can be inherited by offspring and is as Kohout writes, *a blood sister to immunity and they sprout together. When an organism has become immune to a disease, it has also acquired hypersensitivity against a protein (serum)* [11]. Both articles seem to be reviews leaning exclusively on the results of experimental and clinical research of foreign authors. It is understandable, especially if we take into account the fact that before 1920's, there was no original research performed in our country. The beginnings of such research may be traced back to 1920's, to research pioneers and founders of different clinical disciplines, such as internist Ivan Hugo Botteri (1876-1963), pediatrician Ernst Mayerhofer (1877-1957) and dermatovenereologist Franjo Kogoj (1894-1983). Botteri was one of the first to introduce skin test to diagnose echinococcus. Ernst Mayerhofer, Pirquet's student, worked on allergy in children, and Franjo Kogoj was interested in manifestations of hypersensitivity in skin and venereal diseases. The first extensive work on allergy signed by the two contemporaries, Botteri and Mayerhofer, appeared on the pages of *Liječnički vjesnik* already in the twenties and thirties of the 20th

PAPERS ON ALLERGY PUBLISHED IN LIJEČNIČKI VJESNIK 1877-1977

No	Year	Author	Article title
1	1912	Muller O.	A death due to anaphylaxis after the third injection of antidipteric serum
2	1916.	Kohout B.	Anaphylaxis
3	1917.	Buijwid O.	Anaphylaxis
4	1922.	Botteri I. H.	On anaphylaxis by echinococcus
5	1923.	Botteri I. H.	Anaphylaxis in Internal Medicine
6	1923.	Botteri I. H.	On anaphylaxis by echinococcus
7	1923.	Stangl A.	Interesting case of anaphylaxis
8	1925.	Botteri I. H.	On anaphylaxis by echinococcus
9	1927.	Berlot J.	Mechanism of anaphylaxis in blocked animal
10	1928.	Mayerhofer E. Lypolt-Krajanović M.	Erythema toxicum neonatorum-Leiner as a main symptom of allergy of newborn
11	1929.	Mayerhofer E.	Infectious diseases in children and their relation to Pirquet's term of allergy
12	1930.	Mayerhofer E.	News about allergy and the allergic syndrome of newborn
13	1931	Vuletić V.	Diagnosis and therapy of allergies with particular emphasis to hay fever and asthma
14	1932.	Franković V.	Drug hypersensitivity
15	1935.	Winter-Grossmann Š.	Idiosyncrasis against cow milk
16	1940	Kogoj F.	Treatment of drug erythrodermias
17	1940.	Stein B.	Allergic syndrom of intestinal tract and its healing with efedrin and fenilzopropilamin
18	1941.	Banić M.	About allergy
19	1942.	Čajkovač Š.	About so far unknown alergoderma with broncial asthma
20	1946.	Stern P.	About new antihistaminic and their mechanisms
21	1948.	Skrivaneli N.	Syndrom allergicum neonatorum (Mayerhofer)
22	1948.	Grobelnik I., Pansini K.	Penicilin as cause of allergy
23	1949.	Forenbacher G.	Dermatitis caused by streptomycin sensibilization
24	1952.	Marchionini A.	The problem of allergy in Dermatology
26	1953	Milojević B.	Therapy of allergic rhinitis with cortisone
27	1959.	Lopašić R.	Alergie in Neurology
28	1960.	Čupar I.	The importance of allergic manifestations in mouth
29	1962.	Radonić M., Čepelja Z.	Granulocitopeny caused by gralipirin and penicilin anafylaxis
30	1962.	Puretić Š.	The first Congress of allergologists in FNRJ
31	1963.	Mimica M, Babić D., Kohler-Kubelka N., Volarić-Mršić I.	Pollinosis
32	1963.	Horvat Z., Marinšek-Broz V., Čičin-Šain Š.	Gastrointestinal Food Allergy under the Clinical Picture of Malignant Tumour
33	1963.	Kalačić I.	The fifth allergological congress
34	1963.	Vurdelja B.	Pollinosis Calendar
35	1964.	Šakić D.	Allergie Recurrent Palpebral Oedema in Children
36	1965.	Čvorišćec B.	Skin tests in allergic patients
37	1966.	Rumbolt Z, Juretić M.	Basophil Degranulation Test in Penicilin Sensitivity
38	1967.	Krstulović S.	Allergie Recurrent Palpebral Oedema in Children with Eosinophilie Meningitis
39	1968.	Periš Z.	Dermatitis Contacta Allergica Medicamentosa
40	1970.	Krmpotić-Nemanjić J., Keros P.	Allergic Rhinitis
41	1972.	Knežević M.	Therapy of allergic manifestationsof respiratory system with gamma globulin
42	1974.	Kolbas V.	Present day child allergology in Croatia
43	1975.	Kolbas V.	The fifth meeting of allergoslogists of Yugoslavia
44	1975	Kolbas V., Pansini K.	Clinical testing of Indulian in hay fever in children
45	1976.	Kolbas V.	The fifth Congress of allergoslogists
46	1976.	Šrenger Ž	A combined treatment of allergic conjunctivitis
47	1977.	Paleček I.	Clinical evaluation of penicilin hypersensitivity by PPL test

century, whereas the work of their younger colleague, Franjo Kogoj, was published in the same journal in the 1940's and in greater extent in other journals or group publications.

BOTTERI, MAYERHOFER AND KOGOJ – BEGINNINGS OF RESEARCH IN ALLERGOLOGY IN CROATIA

Having finished medical school in Vienna in 1901, Ivan Hugo Botteri spends a year in Vienna clinic of internal medicine and then moves back to Croatia, where he starts working as a physician at the Regional hospital in Zadar in 1902. In 1910, he becomes a *primarius* at the Regional hospital in Šibenik, then Head of the Department of Tuberculosis, and then the hospital director in 1915. In 1922, he arrives to Zagreb, where he assumes the position of Head of Department of Tuberculosis at Sisters of Mercy Hospital. After he was habilitated in 1923, he became a honorary professor of internal propaedeutics at Zagreb University School of Medicine. From 1934 to 1936, he run the Department of Internal Medicine in Foundation Hospital in Zagreb; in 1936, he was elected a full-time professor at Zagreb University School of Medicine and Head of Department of Medicine. Botteri was one of the fathers of internal medicine in Croatia. He published many works in the field of pneumophtysiology, hematology, cardiovascular pathology, gastroenterology, and diagnosis and therapy of Mediterranean subtropical diseases [13].

While he was working in Šibenik hospital, he conducted and published research related to echinococcosis [14,15]. He published his work on sensitization of a man to echinococcal antigen, transfer of the allergy onto another person, and desensitization by intravenous administration of the same antigen in local and international journals [16-19]. As early as 1923, Botteri prepared echinococcal antigen – a preserved hydatid liquid from an echinococcal cyst, which can be used in testing allergies to echinococcus. He was also the first to perform experimental investigation and describe anaphylactic shock caused by echinococcal antigen, otherwise known as *Casoni-Botteri reaction*. He discovered latent eosinophilia as a sign of the existing allergy to echinococcal antigen and tried to use it in therapy. He investigated the mechanism of penetration of echinococcus into bronchi and designed a diagnostic skin test to distomiasis [20].

Botteri's interesting understanding of the notion of allergy and anaphylaxis was published under the title *Anaphylaxis in Internal Medicine* [17]. Here is an excerpt from the article:

So, step by step, we have arrived to the notion of anaphylaxis as we understand it today, which allows us to articulate quite a firm definition despite some exsisting differences in opinion among researchers. Here is the definition: *In capite*, there is the word *allergy*, which quite conveniently does not say anything more than the reactivity of an organism has changed,

without prejudicating anything further. This allergy may be related to antigen and non-antigen substances: the main representatives of allergy, in the latter case, are idiosyncrasies to medications, as forms of hypersensitivity. There is also another form – reduced sensitivity. What interests us here the most is the former form of allergy, the one directed at antigens. In this form of allergy, we have two fundamentally different forms of antigens: toxins, which are primarily toxic, and protein antigens, which are not primarily toxic. In the organism toxins elicit production of antitoxins, which act without a complement; protein antigens, on the other hand, elicit the production of anti-substances, which are active only in combination with complement binding. As a result of antigen-anti-substance reaction, reduced sensitivity (antitoxic immunity) develops typically in the former case, whereas in the latter case, hypersensitivity develops. It is the hypersensitivity to protein antigens that is, *sensu stricto*, called – anaphylaxis. Both forms can be passively transferred. There are atypical forms of this reaction, which cannot be passively transferred, for example, the hypersensitivity to toxin in the former case, and anaphylaxis in the latter case. We are going to deal with atypical anaphylaxis in the strict sense of the word, i.e. with hypersensitivity to protein antigens (anaphylactogens) [17].

During the 20th century, not only did the steady growth of immunologically-mediated diseases attract increasing attention from scientists and clinicians, but the term *allergy* also became widely accepted. In our geographical area, Ernest Mayerhofer, student of the coiner of this word, was a typical representative. His research laid the foundation to scientific research into allergies in pediatrics. He observed that the process of biological allergization of a newborn occurs by constant passage of protein substances by placental blood from the mother to the fetus, especially during the last months of pregnancy. According to Mayerhofer, all biological allergic reactions may be classified into four groups: skin, gastrointestinal, genital, and other benign reactions. The first group comprises *erythema toxicum neonatorum*, (Leiner – Moussous-Mayerhofer). He set the age limit for biological allergization of a newborn to the 42nd day of life, because biological changes in the body of a newborn may appear until that time. All these achievements are included in a chapter titled *Allergic syndrome in newborn – Mayerhofer* in the famous Mayerhofer's pediatric textbook [21]. In that chapter, Mayerhofer explains how his observation of Leiner's rash in newborns, as well as his opinion that the rash is of allergic nature, prompted him to look for one single cause to all similar and, at the time, known phenomena, which partly carried the name "pregnancy reaction". As early as 1925, he announced that many of these phenomena could be present in one and the same newborn simultaneously, appearing gradually one symptom after the other, and

how he often had the chance to establish simultaneous existence of toxic exantema, vaginal bleeding, and swollen breasts [21-23]. Mayerhofer clearly understood that allergic syndrome in newborns included not only *erythema toxicum* as the main symptom of general allergy, but also many other symptoms, such as peripheral swelling of lymph glands, hypereosinophilia, and initial leukopenia with leukopenic tumor of the spleen (*Mayerhofer-Lemež*). His list of symptoms also included the reactions of smooth muscles, transitory diarrhea, traces of blood in the stool or even melen, bleeding from the uterus, famous "pseudowasserman's reactions," and some particular characteristics of vaccine reaction in newborns [21-24]. What seemed different was the same, and Mayerhofer managed to raise interest in research in the field of allergies and gather a group of young investigators. He himself stated that further improvement of allergic symptomatology in newborn occurred due to research performed by his student, L. Lemež, who found absolute eosinophilia in the blood of newborns and allergic leukopenia analogous to leukopenia in serum disease, which was first found by Bianka Bienenfeld [23]. The list of researchers later included the names of Niko Skrivanelli, Mayerhofer's successor, internist Vinko Vuletić, and pediatrician Branko Dragišić [24].

The third stronghold in the development of allergology in our geographical area doubtlessly belongs to Franjo Kogoj (1894-1983), Head of the Clinic for Skin and Venereal Diseases in Zagreb (1927-1965). It was due to his efforts that the laboratory for allergies, an outpatient office for professional diseases, and a laboratory for medical biochemistry were established in 1948 at the Clinic. Albin Brnobić whose later interest was especially dedicated to allergies became their first Head [25,26]. Kogoj introduced a *critical moment* as an objective criterion for evaluation of the time needed to cure syphilis and superimposed epidermal reaction as a diagnostic method. He gave a final definition of Mljet disease and introduced a histological novelty in the world dermatovenereologic literature – *spongiform pustule* [27-29]. More about his role within the development of allergology in Croatia can be found elsewhere.*

ALLERGOLOGY RESEARCH: OPENING COMMUNICATION AMONG DIFFERENT MEDICAL FIELDS

In addition to serum sickness, anaphylaxis was rapidly implicated in the pathogenesis of hay fever (1906), urticaria (1907), food idiosyncrasies and eczema (1908), asthma (1910), hypersensitivity to aspirin (1912), reactions to bee stings (1914), and a variety of non-specific clinical manifestations. The impact of this was to stimulate the recognition and aggregation of a constellation of previously disparate conditions increasingly referred to as *allergic disorders*, in which the clinical and pathological manifestations were generated by the patient's immunological (allergic) idiosyncrasies rather than by the allergen itself [2].

Accordingly, if we follow the course of the development of allergology on the basis of the titles of articles published in *Liječnički vjesnik*, we can see that, in the early 1930's, authors wrote on diagnosis and therapy of asthma and pollenosis, hypersensitivity to medications, and sensitivity to food. As the administration of specific therapy, especially antibiotics, became a regular practice in 1940's, the number of reports of hypersensitivity to medication, especially to penicillin, started to increase (Table 1). A line of thinking linked to the public health movement of the period aimed to incorporate questions of employment and life style thus professional diseases were on their way to establish a place for themselves. Professional allergic dermatosis attracted interest of variety of physicians such as Dušan Jakac who pioneered in founding dermatovenerology as an academic discipline in Rijeka [30,31]. Having in mind current problems and hazards of industrialization Andrija Štampar then president of Yugoslavian Academy of Sciences and Arts (YASA) proposed the foundation of Institute for Industrial Hygiene in 1947 later called Institute for Medical Research and Occupational Health in Zagreb, whose activities have been directed towards study of effects of chemical and physical agents upon human health. In the beginning its work was mostly concentrated on the effects of occupational exposure on worker's health but later, it gradually expanded to studies of environmental exposure of the general population [32]. Among the earliest publications considering occupational hypersensitivity which appeared in *Liječnički vjesnik* was one written by Tihomil Beritić describing the relationship between hypersensitivity to flour and chronic bronchitis in bakers [33].

In 1950's, the interest in allergy spread across many other fields of medicine and there was almost no clinical or other area where, at the time, allergology research was not performed or at least a case report published. It was in those times that he need arouse for synthesizing knowledge and opening communication among different medical fields. In result and upon Kogoj's initiative, the Yugoslav Academy of Science and Arts organized a symposium on allergy in 1951. The symposium lectures were published in the works of YASA the following year [34], when the Allergology Section of Croatian Medical Association was founded as well, with Franjo Kogoj elected the president and Štefanija Puretić the secretary [35].

The fact important for the development of allergology in Croatia was that Drago Ikić had run the newly-opened Department for Control and Testing of Sera and Vaccines. Actually when the Institute for Control and Testing of Immunologic Preparations and Serovaccination Institute were separated from the Central Hygiene Institute, Ikić became the director of the former (by the Decision of the Council of the Public Health dated May 16th 1956) and director of the latter (by the Decision of the Council of the Public Health dated Jun 25th 1956) [36]. Serovaccination Institute changed the name into Institute of Immunology in 1961 and merged with the Institute for Control and Testing of

Immunobiological Preparations in 1969. Drago Ikić was directing the Institute of Immunology for 21 years, from 1961-1982 [37]. In these institutions, a special attention was paid to the production of allergens for testing and desensitization, a procedure first performed by Neda Keler-Kubelka, who earned her PhD degree in 1964 by defending the thesis titled, *Testing of pollen allergens by different immunologic methods and analysis of the value of these methods for standardization of allergens*, and published a series of works in this field [37]. In the same year another PhD thesis was completed in this field by Dragan Dekaris entitled *The Effect of antiserum antibody injection in rats*. Further on Dragan Dekaris has successfully applied the results of basic into clinical immunology and allergology [38], has established the Croatian Referral Center for Clinical Cellular Immunodiagnostic [39], and published the first book on basic allergology [40]. During different periods he obtained the role of Head of Section (1971-1982), Head of Department (1982-1992), Scientific Director (1992-1994), Vice-Director (1994-1997), Head of Department for Research and Development of the Institute of Immunology. Generally, the Institute of Immunology offered a strong support to the technological and research development in the field of allergology. At the same time, it provided education and stimulation to a long range of experts specially interested in allergology, as it does so today.

The spread of interest in allergology influenced its penetration into different medical fields during the second half of the 20th century, and specialized units for allergology were being opened within Croatian hospitals as well as research and medical centers. In 1958 in Dubrovnik (Lapad) the Climatic Centre for recovery and curing of the respiratory diseases was opened. In the beginning only one physician Željko Radić was in charge of making intra-cutane tests on inhalation and nutritive allergens, but this early beginnings of allergology in Dubrovnik were followed up by work of Nikša Sindik and Ivo Kalačić in the later periods. In the 1962 the special hospital for allergic diseases was established in Dubrovnik regularly supervised by reputable experts of the field Milorad Mimica and Tihomil Beritić. In its laboratories tests on group and specific antigens as well as provocation tests were performed. Very noticeable is the foundation of the Pulmology and Allergology Unit of Dr. Josip Kajfež Hospital Internal Clinic, established by internist and one of the prominent pioneers of allergology Milorad Mimica in 1963. Its first Head was Branimir Čvorišćec succeeded later by Ivan Paleček and Zdenko Tudman. The Unit was the leading institution in Croatia oriented in development of clinical allergology as well as *in vitro* and *in vivo* diagnostics and therapy. In its immunological laboratory established by Neva Sket-Janković the first IgE antibody test was completed in this part of Europe as well as the first biological standardization of allergens and RAST inhibition [41,42]. In the Institute for Protection of Mother and Child, Head of Allergology Department was a pediatrician, Vladimir Kolbas, whose interest lay in syndrome of agammaglobulinemia, respiratory allergies in childhood,

immunoallergic aspects of idiopathic pulmonary hemosiderosis, dynamics of neuro-vegetative response in atopic diathesis in children. We also have to mention the work of the founder of the Department of Immunology and Head of Pediatric Pulmonary Department at Zagreb University Hospital Center, Drago Buneta.

Allergies gradually attracted interest even of physicians in surgical specialties, like Ear, Nose and Throat specialists Željko Poljak and Zvonimir Krajina at the ENT Clinic of Zagreb University School of Medicine. Željko Poljak's earned his PhD degree in the field of allergology, his PhD thesis titled *Rhinitis Allergica and Infection of Nasal Mucosa* in 1969, and the topic of his habilitation lecture that he held at Zagreb University School of Medicine in 1971 was *Problems of Aerosol Therapy in Rhinology*. He was the Secretary of the Association of Allergologists of Yugoslavia and the Secretary of the Allergology Section of the Croatian Medical Association. There was almost no medical discipline where the names of researchers in the field of allergology could not be found. Their work certainly deserves much deeper attention, but it would go much beyond the scope of the present article.

In 1961, the congress of allergologists of Yugoslavia took place in Zagreb; in 1962, a professional meeting of allergologists of Yugoslavia was held in Dubrovnik; and in 1964, followed an International Symposium in Allergology in Zagreb, Split, and Hvar. In the fall of 1964, at the 3rd Congress of Croatian Physicians held at "Dr Josip Kajfeš" hospital and Clinic of Dermatovenereology in Zagreb, the first seminar in allergology was held under the title "Basics of clinical allergology". The seminar was attended by 25 participants. In 1966, Zagreb branch and Allergology Section of the Croatian Medical Association held a panel discussion on *Allergy for Practitioners*. In 1967, Allergology Section of Croatian Medical Association also organized counseling of allergologists in Croatia at the republic level, and in May of the same year, a symposium on allergic diseases of respiratory system on the island of Hvar, and since 1973, a journal *Glasilo alergološkog centra JAZU* (The Journal of Allergology Center of YASA) has been published in Hvar. We also have to mention the establishment of health care institutions for treatment of allergic diseases at the Adriatic coast, in Dubrovnik, Veli Lošinj, Hvar, and Baška Voda [35].

Liječnički vjesnik used as a main source for interpretation of allergology development in Croatia is not, however, representative source of the allergology development for the whole analyzed time period particularly for the reason that since 1960-ies large part of Croatian immunologists and allergologists started to publish the best part of their results within various international publications. The second half of the 20th century witnessed a strong takeoff in the field of allergology, a medical field that had clearly defined its scope and goals in the previous decades and established a fruitful cooperation between basic and clinical research. In Croatia those developments have gained a considerable

scientific importance since second half of the 20th century particularly when the work of Nikša Alegretti, Borislav Nakić and Vlatko Silobrić have gained a considerable scientific importance and become internationally well-recognized (44-47). In 1970's, the phenomenon of immunity took the central place in biomedicine and became a meeting point of cell biologists, immunochemists, genetics, and molecular biologists, announcing a new stage in research and development of allergology.

References

- [1] Porter R. Porter R. The greatest benefit to mankind. New York, London: W.W. Norton&Company; 1999.
- [2] Jacksom M. Allergy and History. Stud. Hist. Phil.&Biomed.Sci. 2003; 34: 383-98.
- [3] Berlot J. Razvitak mikrobiologije i imunologije u Hrvatskoj do 1923.god. U: Topolnik E. (ur) I simpozij o historiji mikrobiologije i imunologije u Hrvatskoj do 1923.godine. Zagreb: JAZU 1973.
- [4] Belicza B. Od proizvodnje animalnog cjepiva za zaštitu od velikih boginja do proizvodnje prvih humanih virusnih cjepiva, antibakterijskih preparata i antitoksičnih seruma, te osnivanje prvog odjela za proizvodnju lijekova biološkog podrijetla u zagrebu. U: Vranešić B. (ur) Od «Kraljevskog zemaljskog zavoda za proizvodjanje cjepiva proti boginjama» do Imunološkog zavoda Zagreb 1893-1993. Zagreb: Imunološki zavod; 1993.
- [5] Pirquet C., Schick B. *Serumkrankheit*. Leipzig: Deuticke 1905.
- [6] Miller O. Slučaj smrti uslijed anafilakse iza trećega uštrcavanja antidiftericnoga surutka. Liječ Vjesn 1912;34:55.
- [7] Silverstein AM. Clemns Freiherr von Pirquet : a pediatrician explains immune complex disease in 1906.
- [8] Gutschy Lj. Pregled novijih radnja i teorija u nauci o imunitetu. Liječ Vjesn 1908; 30:335-38.
- [9] Bubanović F. Savremeno stanje imunokemije Liječ Vjesn 1913;35:214.
- [10] Stangl A. Zanimiv slučaj serum anafilaksije Liječ Vjesn 1923;45:369.
- [11] Kohout B. Anafilaksija Liječ Vjesn 1916;38:4-12.
- [12] Bujwid O. Anafilaksija Liječ Vjesn 1917;39: 347
- [13] Dugački V. Botteri Ivan Hugo. Zagreb: Jugoslavenski leksikografski zavod «Miroslav Krleža» 1989. sv. 2 str. 215-16.
- [14] Botteri I. H. O mehanizmu prodora plućnih ehinokokovih cista u bronhe. Liječ Vjesn 1938;60:1-3.

* The paper on Franjo Kogoj will be given by professor Jasna Lipozenčić, pp 53-66

- [15] Botteri I. H. O nekim problemima endemične ehinokokoze kod nas. Liječ Vjesn 1953;75:99-101.
- [16] Botteri I. H. Referat prethodnog izvješća o ehinokokovoj anafilaksiji. Liječ Vjesn 1922;44:64-67.
- [17] Botteri I. H. Anafilaksija u internoj medicini Liječ Vjesn 1923;45:230-31.
- [18] Botteri I. H. O ehinokokovoj anafilaksiji Liječ Vjesn 1925;47:185-196.
- [19] Botteri I. H. Serologija ehinokokove bolesti 1930;52:591-610.
- [20] Botteri I. H. Distomatoza. Liječ Vjesn 1935;57:196-99.
- [21] Mayerhofer E, Dragišić B. Pedijatrija (II izd.). Zagreb: Nakladni zavod Hrvatske 1948; p. 321.
- [22] Mayerhofer E. Općenitosti o infekcijskim bolestima djeteta i njihov naročiti odošaj prema Pirquetovom pojmu alergije. Liječ Vjesn 1929;51:410-433.
- [23] Mayerhofer E. Nove radnje o alergiji i moj alergični sindrom novorodjenčadi. Liječ Vjesn 1930 52:345-49.
- [24] Skrivaneli N. Syndrom allergicum neonatorum (Mayerhofer). Liječ Vjesn 1948;70: 3-9.
- [25] Lipozenčić J, Čikeš N, Marinović B. (ur.) Katedra za dermatovenerologiju Medicinskog fakulteta Sveučilišta u Zagrebu. Medicinski fakultet Sveučilišta u Zagrebu: Zagreb; 2006.
- [26] Fatović-Ferenčić S. The Chair of Dermatovenerology at Zagreb School of Medicine. On the occasion of the 80th anniversary of its foundation (1921-2001). Acta Dermatovenerologica Croatica 2001;9:267-273.
- [27] Holubar K. Franjo Kogoj and the spongiform pustule. Am J Dermatopathol 1985; 7:191-195.
- [28] Dobrić I, Miljević A et al. Psoriasis Pustulosa: Classification and Light Microscopy. Seventy Years of Kogoj's Spongiform Pustule: 1927-1997. Psoriasis 1997; 39: 6-9.
- [29] Fatović-Ferenčić S, Holubar K. Mal de Meleda: From legend to Reality. Dermatology 2001;203:7-13.
- [30] Fatović-Ferenčić S. Dušan Jakac. Hrvatski biografski leksikon Zagreb: Leksikografski zavod «Miroslav Krleža» 2005. sv. 6 p. 240-41.
- [31] Fatović-Ferenčić S, Lipozenčić J. Dušan Jakac: The Life in Dermatovenerology. Acta Dermatovenerologica Croatica, 2003;11:35-38.
- [32] Reiner E, Banić N. Institute for Medical Research and Occupational Health Zagreb 1948-1993. Zagreb: IMI; 1994.
- [33] Beritić T, Valić F. O odnosu preosjetljivosti na brašno i kroničnog bronhitisa u pekara. Liječ Vjesn 1971;93:991-96.
- [34] Kogoj F. (ur) Simposion o alergiji. Zagreb: JAZU; 1952.
- [35] Mimica M. Alergološka sekcija. U: Keros P. (ur) Zbornik Liječnika Hrvatske; Zagreb: ZLH 1974.

- [36] Archive of the Institute for the History of Medicine of the Croatian Academy of Sciences and Arts – original document donated by Drago Ikić.
- [37] *Pende B.* Prilog novijoj povijesti Imunološkog zavoda. U: Vranešić B. (ur) Od „Kraljevskog zemaljskog zavoda za proizvodjanje cjepiva proti boinjama „ do Imunološkog zavoda Zagreb 1893-1993. Zagreb: Imunološki zavod 1993; p 73.
- [38] *Fauve R. M., Dekaris D.* Macrophage spreading: Inhibition in delayed hypersensitivity. *Science* 1968;160:795-96.
- [39] *Dekaris D.* Founding and activity of the Croatian Referral Center for Clinical Cellular Immunodiagnosis. *Rad Hrvatske akademije znanosti i umjetnosti* 1994; 466:51-64.
- [40] *Dekaris D.* Temeljna alergologija. Zagreb: Školska knjiga 1983.
- [41] *Paleček I, Čvorišćec B, Lipozenčić J.* Povijest internističkih struka u Hrvatskoj. Zagreb: AMZH 1998;95-105.
- [42] *Bušić M.* Opća bolnica Sveti Duh. Zagreb : Opća bolnica “ Sveti duh”; 2006.
- [43] *Poljak Ž.* Doprinos otorinolaringologa alergologiji i kliničkoj imunologiji. U: *Krajina Z, Poljak Ž.* (ur) Otorinolaringologija u Hrvatskoj. U povodu 80. obljetnice osnutka prve ORL klinike u Hrvatskoj. Zagreb: Klinika za otorinolaringologiju KBC-a u Zagrebu; 2001, pp. 107-8.
- [44] *Silobrić V.* Forty years of Transplantation immunology in Croatia. *Period biol* 97:167-170.
- [45] *Silobrić V.* Boris Nakić začetnik transplantacijske imunologije u Hrvatskoj: Liječ vjesn 1982;104:140-142.
- [46] *Dekaris D.* Nikša Alegretti (1920-1982) U: Ikić D. (ur) Spomenica preminulim akademikima vol. 21, Zagreb: JAZU 1984, p.39.
- [47] *Dekaris D, Čulo F.* (ur) Klinička imunologija u nas. Zagreb :JAZU&Naprijed 1990 pp. XXVI and 590.
- [48] *Rabatić S.* Croatian Immunological Society – 30 years of organized activities. *Immunology Letters* 2000;73:23-7.

Sažetak

Početak i razvoj alergologije u Hrvatskoj u razdoblju od 1870. do 1970. godine

Razumijevanje podrijetla alergije kao imunološkog i eksperimentalnog fenomena, odvijalo se na području Hrvatske slično kao i u bliskim europskim zemljama. Najvažniji događaji i otkrića prikazani su u ovom radu za razdoblje 1870-1970. godine.

Pokazalo se da su ključni momenti razvoja alergologije u nas, isprva bili vezani uz proizvodnju cjepiva te nastanak Imunološkog zavoda i njegovih preteča, tijekom prve polovice 20. stoljeća. Utemeljenje ovih ustanova predstavljalo je čvrstu institucijsku vertikalu presudnu za razvoj alergologije i imunologije u Hrvatskoj sve do danas. Raščlambom radova objavljenih na stranicama Liječničkog vjesnika za razdoblje 1877-1977. prezentirana je zastupljenost alergoloških tema te uloga pojedinih najznačajnijih ličnosti. Ovi radovi, međutim, ne daju potpunu sliku alergologije u Hrvatskoj osobito od 60. godina 20. stoljeća nadalje, kada naši autori veći dio svojih istraživanja objavljuju u međunarodnim časopisima. Istaknuta je uloga interniste Ivana Huga Botterija (1876-1963), pedijatra Ernesta Mayerhoferera (1977-1957) i dermatovenerologa Franje Kogoja (1894-1983), koji su svojim radom utrli put znanstvenoistraživačkom radu te uspješno poticali interes za ovo područje.

Objedinjavanje interesa za alergologiju stručnjaka različitih medicinskih specijalnosti rezultiralo je osnutkom Alergološke sekcije Zbora liječnika Hrvatske 1952. godine. Ova je udruga odigrala daljnju važnu ulogu osobito u edukaciji šire akademske zajednice organizacijom simpozija, kongresa i seminara.

Pokazalo se da je rad institucija i liječnika tijekom prve polovice 20. stoljeća, dao čvrst temelj suvremenim istraživanjima, oblikovanju subspecijalnosti te nastanku alergoloških centara i jedinica unutar različitih medicinskih institucija diljem Hrvatske.

Ključne riječi: Povijest medicine – Hrvatska; razvoj alergologije – Hrvatska; Liječnički vjesnik – alergija; 19. stoljeće; 20. stoljeće

** Acknowledgement: I appreciate help of Professors Drago Ikić, Dragan Dekaris, Branimir Čvorišćec, Željko Poljak and Nikša Sindik

*** This research was part of the project Croatian medical identity and its European context supported by the Ministry of Science and Technology of Republic of Croatia.

ACADEMICIAN FRANJO KOGOJ AND ALLERGOLOGY IN CROATIA

Jasna Lipozenčić, †Albin Brnobić

University Department of Dermatology and Venereology,
Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia

Summary

Franjo Kogoj was regular member of the Yugoslav Academy of Sciences and Arts (1947), first secretary and Vice-President elect for several terms of office of the Medical Science Section of the Yugoslav Academy of Sciences and Arts (1958-1973), long-standing head of University Department of Dermatology and Venereology, Zagreb University Hospital Center, and head of Department of Dermatology and Venereology, Zagreb University School of Medicine. Numerous papers by academician Kogoj address allergic events in the body and their role in the disease course and morphology, prognosis, prophylaxis and management. He wrote about experimental studies of allergic reactions in eczema and pruridermatitis, a term he introduced in dermatologic terminology to replace the terms neurodermatitis, endogenous eczema, prurigo-asthma and Besnier's prurigo. He tackled the issues of allergy in many papers, always bringing something new in the field. The concepts elaborated in his paper entitled "Ein Vierteljahrhundert Vasculitis", presented at the Deutsch-japanischer Angiologien Kongress held in Essen in 1973, were received with high interest. In 1965, the Center of Allergology of the Yugoslav Academy of Sciences and Arts on the island of Hvar was established on his initiative, first at premises adapted for this purpose, then moved to a modern building consisting of inpatient and outpatient departments, diagnostic laboratories of biochemistry, pulmonary function testing, electrocardiography, radiology, physiotherapy, and library in 1973. F. Kogoj and A. Brnobić were founders of the Section of Allergology of the Croatian Medical Association (1952), as an attempt at unifying the work of medical professions dealing with allergic diseases. Kogoj published about 230 scientific and professional papers, 70 of them addressing allergology and immunobiology issues; 5 books; several monographs; and valuable contributions in a number of handbooks and textbooks.

Correspondence to: Professor Jasna Lipozenčić, MD, PhD, University Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Šalata 4, 10000 Zagreb, Croatia, e-mail: jasna.lipozenic@zg.htnet.hr

A brilliant era in the history of the Zagreb University School of Medicine and Croatian Academy of Sciences and Arts ended with F. Kogoj.

Key words: *Academician Franjo Kogoj and allergology; Pruridermatitis allergica chronica constitutionalis; Secretary of Medical Science Section of Yugoslav Academy of Sciences and Arts*



Professor Franjo Kogoj, MD, PhD, University Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine Zagreb, from 1927 till 1965

FRANJO KOGOJ IN CROATIAN MEDICINE

Department of Medical Sciences of Yugoslav Academy of Science and Arts (YASA) has published Testimonial to the Late Academicians – Volume 28, 1985, dedicated to Franjo Kogoj, regular member of Zagreb YASA, presented at Commemorative Ceremony held on October 1, 1984 at YASA Home. Professor Sead Midžić, YASA associate member, wrote: "Academician Franjo Kogoj departed this life peacefully, quietly and gently, in acquiescence of harmoniously completed life, in his native place of Kranjska Gora, to which he had always been coming back. He was long-standing head of University Department of Dermatology and Venereology, and of School of Medicine Department of Dermatology and Venereology, dean and vice-dean of the Zagreb University School of Medicine for several terms of office, first secretary of YASA Department of Medical Sciences, and its elected vice-chairman for several terms of office (1958-1973). A brilliant era in the history of our School of Medicine and our medical sciences has terminated with his departure. We have lost him; however, he has bequeathed to us his great opus and

example that will always stimulate us to follow him as an ideal and an inexhaustible source of inspiration" [1]. He was elected regular YASA member in Zagreb in 1947. Kogoj devoted his life to the research, education and clinical work in the field of allergology and clinical immunology in dermatology and venereology [2].

Franjo Kogoj was born on October 13, 1894 in Kranjska Gora, Slovenia, where he died on September 30, 1983. He studied at Charles University School of Medicine and graduated from it in 1920. Then he served residency at University Department of Dermatology and Venereology in Prague, headed by Prof. Šamberg who offered him the position of assistant. In 1923, Kogoj stayed at University Department of Dermatology and Venereology in Brno, headed by Prof. Tryba, then at University Department of Dermatology and Venereology in Zagreb as assistant to Prof. P. Šavnik. As early as 1924, when Prof. Šavnik died, heading of the Department was taken over by Kogoj [1,2]. During the 1924-1926 period, he received training at large departments with Prof. A. Tryba (Brno), Prof. J. Jadassohn (Wrocław), Prof. Pautrier (Strasbourg) and Prof. A. Sézary (Paris). In 1925, he habilitated as private Assistant Professor of Dermatology and Venereology at Masaryk University in Brno, with a thesis entitled "On sclerodermas and scrofulodermas" [1-4]. He found his scientific interest at Prof. Šamberg's Department in Prague, where he mastered precision, systematic approach and morphology of skin lesions. Prof. Jadassohn aroused his interest in experimental studies he had dealt with throughout his professional life (e.g., dermatomycoses, pyodermas, syphilis, tuberculosis, etc.). Specific inflammation and morphogenesis of the pathologic substrate consequential to tissue sensitization led him to allergic phenomena. These considerations and presentation of allergy topics pointed to Kogoj's work as a scientist and established his place in science [2].

Kogoj was a versatile scientist. He entered the European and world's scientific legacy by his clinical and scientific studies, in particular his histological discovery of the "spongiform pustule of Kogoj" in the diagnosis of pustular psoriasis. He contributed to the internationalization of Meleda disease (*mal de Meleda*), based on his epidemiological studies and histological verification of this genodermatosis. He was author of the film "Mal de Meleda" that was first released at XII International Congress of Dermatology in Washington, 1962 (4). Dorland's Illustrated Medical Dictionary, published by W. B. Saunders, Philadelphia, 1974, brings the entry "Kogoj's pustule: spongiform pustule of Kogoj" [1].

Kogoj advocated completion of the Department premises at Šalata, Zagreb, and University Department of Dermatology and Venereology was opened on December 9, 1928. In 1927, Kogoj was appointed professor and head of Department, which position he held until retirement in 1965, with interruption during World War II. From 1965 until 1980, Kogoj visited the Department daily, always ready to offer his valuable professional

assistance from his rich and inexhaustible treasury of knowledge [4]. At Department opening ceremony, he held a lecture entitled "On the causal problem in dermatology and in medicine in general". Academician Kogoj said he was happy to speak in Zagreb as an outstanding cultural center, and he would invest all his efforts for the Department activities to contribute to the honor and reputation of the Croatian people both at home and abroad. Academician Kogoj performed this solemn promise honorably and completely through his numerous papers [4-11].

The Ljubljana University School of Medicine appointed him scientific and educational head of the University Department of Dermatology and Venereology during the 1955-1965 period. He served the position of dean in the academic years 1933-1934, 1946-1947, 1948-1949 and 1951-1952, and of vice-dean in the academic years 1952-1953 and 1953-1957. With his work, Academician Kogoj upgraded both Zagreb and Ljubljana University Departments of Dermatology and Venereology, the former being among the leading ones in Europe.

Kogoj continued his activities as director of the Institute of Clinical Medical Research, School of Medicine and Zagreb University Hospital Center from 1967 until 1974. He published about 230 papers in scientific and professional journals, periodicals, handbooks and textbooks, along with eight books and monographs (1-4). Especially prominent are his papers on the research into hereditary dermatoses, pyodermas, dermatomycoses, allergic diseases, keratodermas and management of skin diseases, syphilis in particular [2].

Kogoj was the first to use tests for assessment of allergic phenomena, was engaged in the study of endemic syphilis, Meleda disease, and introduced a new therapeutic procedure in the treatment of sporadic and endemic syphilis, thereby emphasizing the so-called critical moment, which he considered as an objective criterion on evaluating definitive cure in syphilis. Kogoj said: "We feel the unavoidable necessity of confirming the critical moment by a negative liquor finding because in early cases only such a procedure permits an immediate therapeutic intervention corresponding to the found condition and giving much more hope of success as that only later time" [12]. Of great importance are his papers on the genesis of pustular efflorescence, psoriasis, atrophoderma, dyskeratosis, scleroderma, pyoderma, eczema and eczematiform dermatoses, exanthema localization, and bullous dermatoses. He distinguished anergic, allergic-hyperergic and positive anergic forms on the basis of tuberculin reaction.

Kogoj's educational and scientific work was abundant in original ideas and procedures, for which he was recognized as one of the leading dermatologists worldwide. He was member of the Standing Expert Council for Therapy Issues of the Federal Healthcare Institute and of the Federal Council for Coordination of Scientific Activities; chairman of the Committee for Medical Sciences and of the Expert Group for Dermatology, Venereology and Allergology; member of the Science Council of the Socialist Republic

of Croatia (SRC); chairman of the Association of Institutions and Organizations for Medical and Pharmaceutical Research in SRC; delegate of the Yugoslav Council of Academies to the Committee for Science and Technology Coordination; member of the Executive Committee of the Board for the Foundation of the School of Medicine in Rijeka; member of Deutsche Akademie der Naturforscher Leopoldina and New York Academy of Science; and corresponding member of the Slovenian Academy of Science and Arts, Serbian Academy of Science and Arts, Bosnia and Herzegovina Academy of Science and Arts, Schweizerische Akademie für medizinische Wissenschaften and Österreichische Akademie der Wissenschaften. He was chairman, honorary member, corresponding member or regular member of forty international and Yugoslav dermatological and medical societies and professional-organizational bodies [2-4].

Kogoj was invested with a number of high federal orders. He received the Federal Governmental Award for his textbook *Dermatološka propedeutika* (Dermatologic propedeutics, 1949); SRC Life Achievement Award (1962); AVNOJ Award (1966); and a number of other certificates of merit. He received the title of *Doctor honoris causa* on four occasions in 1962 at Graz University; honorary doctoral degree in medical sciences in 1961 at Ljubljana University, in 1968 at Zagreb University, and in 1970 at Academy of Military Medicine in Belgrade [1-4].

The great contribution of Academician Kogoj to Croatian dermatology and venereology, allergology in particular, is inarguable.

THE HISTORY OF ALLERGOLOGY IN CROATIA WITH ACADEMICIAN KOGOJ'S INITIATIVE

A detailed account is given of the history of allergology in Croatia as directly related to Academician Kogoj. In 1909, Prof. Ernst Mayerhofer drew attention to and with his coworkers wrote in 1928 in *Liječnički vjesnik* (Lijec Vjesn) about the role of cow's milk allergy in infants and children. Ivan Hugo Botteri was among the first in the world to introduce cutaneous testing in the diagnosis of echinococcosis. These developments and those contributed by Prof. Kogoj made the basis of allergology in Croatia.

Besides numerous papers in dermatology and venerology, Prof. Franjo Kogoj also earned his place in allergology and clinical immunology. His papers *O alergologiji* (On allergology); *L'allergie, cest la vie* (Allergy, that's life) from 1926; *Dijagnostika alergijskih bolesti* (The diagnosis of allergic diseases); and those on drug allergy, urticaria, strophulus, prurigo and allergoderma were major scientific contributions in the field of allergology [13]. Prof. Vuletić contributed greatly to Croatian allergology with his papers on the diagnosis and therapy of bronchial asthma and pollenosis (Lijec Vjesn 1931-1934). In 1948, Prof. Kogoj together with Prof. Brnobić opened Allergology Unit and Outpatient

Clinic for Occupational Diseases at University Department of Dermatology and Venereology in Zagreb. In 1956, on the initiative of Academician Kogoj, Prof. Jakac established Laboratory of Allergology at Braća Sabol University Department of Dermatology and Venereology in Rijeka, and in 1957 Prof. Vukas opened Division of Allergology at Dr. Z. Kučić University Department of Dermatology and Venereology in Rijeka.

On the initiative of Academician Kogoj, YASA organized Symposium on Allergy and published the papers presented in 1952. Along with the editor F. Kogoj (dermatology and venereology), the experimental aspects of allergy were addressed by Petar Sokolić, endocrine system by Vuk Vrhovac, bronchial asthma by Vinko Vuletić, allergy and digestive tract by Arpad Hahn, tuberculosis by Stanko Ibler, allergy in neurology by Radovan Lopašić, ENT by Srećko Podvinec, dental medicine by Ivo Čupar, and occupational allergic diseases by Branko Kesić.

In 1952, also on the initiative of F. Kogoj, Section of Allergology with dozens of members was established at Croatian Medical Association (CMA). F. Kogoj was the first Section chairman and was later elected life honorary chairman [13].

In 1956, the manufacture of testing and desensitization allergens was initiated at the then Serovaccine Institute (later Institute of Immunology). The International Symposium of Allergology was held in 1964 in Zagreb, Split and Hvar, organized by YASA in collaboration with the CMA Section of Allergology. The European Climatic Center for Treatment of Allergic Diseases was founded on the island of Hvar, on the initiative of F. Kogoj and Grga Novak as YASA chairman. In 1961, members of the CMA Section of Allergology organized the First Congress of Yugoslav Allergologists, with F. Kogoj as president of the Organizing Committee.

The First Convention of Yugoslav Allergologists was held in 1962 in Dubrovnik [13]. Section of Allergology continued its activities in the time to come, including its engagement in the Association of Yugoslav Allergologists. Jubilee celebration of the 40th anniversary of the foundation of the Section of Allergology entitled *Allergologia Croatica 1952-1992* was held in October 1992 at Croatian Academy of Science and Arts (CASA) under the auspices of the CASA Department of Medical Sciences. Plenary lectures on Academician Kogoj and Prof. Mayerhofer paid due reverence to these greats of allergology. Some of the papers presented at the meeting have been published in *Rad Hrvatske akademije znanosti i umjetnosti, Medicinska znanost*, Vol. XXVII, Zagreb, 1994:466 [13].

FRANJO KOGOJ IN ALLERGOLOGY AND CLINICAL IMMUNOLOGY AS APPLIED IN DERMATOLOGY AND VENEREOLOGY

Many Kogoj's papers deal with allergic events in the body and their impact on the morphology and course of disease, its prognosis, prophylaxis and treatment [14-52].

These include papers on anticutins and procutins, superimposed tuberculin reactions, superimposed epicutaneous reactions, superimposed tuberculin-trichophytin reactions, and experimental studies of allergic reactions in eczema and pruridermatitis; he introduced the latter term in dermatologic terminology instead of neurodermitis and endogenous eczema, prurigo-asthma and prurigo Besnier [4]. In many of his papers, Kogoj tackled the issues of allergy, always bringing some new concepts, as allergy was his favorite field of interest [11]. He described numerous intriguing observations in the field of dermatology and elaborated in detail the issue of symptom complex in dermatology; he thoroughly investigated particular syndromes and semi-syndromes, and the issue of pruritus as a symptom. In 1962, he wrote the chapter on urticaria, strophulus, prurigo and pruritus in the complementary part of Jadassohn's *Handbuch der Haut*.

Throughout his professional life, Kogoj was intensively engaged in scientific research up to 1980, as evidenced by the great number of his papers (about 50) published upon retirement. In these papers, he continued dealing with his favorite topics of medicamentous exanthema, medicamentous dermopathies, eczema, pruridermatitis (atopic dermatitis), pruritus, superimposed trichophytin reactions, effect of serotonin on allergic skin tests, allergic events on the skin, so-called corticotropin therapy in dermatology, and role of histamine in eczematous reaction. His latest concepts elaborated in the paper entitled *Ein Vierteljahrhundert Vasculitis*, presented at the Deutsch-japanischer Angiologenkongress held in 1973 in Essen, aroused high interest indeed [3,4,52]. Kogoj published his papers in Croatian, Slovenian, Czech, Polish, Bulgarian, German, French, Italian and English languages, thus being recognized and known all over the world. He mostly wrote his papers alone and some in collaboration with his coworkers Šavnik, Tryba, Jadassohn, Bošnjaković, Farkaš, Franković, Žuka, Ante Vuletić, Gušić, Štefanija Puretić, Milavec, Brnobić, Gizela Luković, Fettich, Zambala, Schwarzwald, Žmegač, Davor Perović and Verena Kogoj-Bakić. He tackled allergy topics in *Problematika ekzema* (The problems of eczema) and *Bolesti kože 1. i 2. dio* (Skin diseases, Parts 1 and 2; 1970 and 1971), a textbook for physicians and students; these books he wrote in collaboration with his coworkers. With these books, Kogoj completed his trilogy (*Dermatološka propedeutika, Spolne bolesti and Bolesti kože*), where he elaborated allergology, overall dermatology and venereology, along with dermatology and venereology propedeutics [2,4].

Owing to his numerous scientific and professional papers, Kogoj was member of editorial boards of a number of renowned scientific and professional journals, i.e. 5 domestic and 12 international journals. At professional conventions, he presented more than 100 case reports, and took active part in vivid discussions at congresses and conventions in Croatia and abroad. He was cited in a number of papers published in *Liječnički vjesnik*, *Zentralblatt für Haut- und Geschlechtskrankheiten*, *Excerpta Medica*, etc., reviewed many books in domestic and international periodicals, wrote congress re-

ports, worked on Medical Encyclopedia and other encyclopedic editions. He was Editor-in-Chief of the following publications: *Izviješća Dermatovenerološke klinike u Zagrebu* (1952); *Simpson o alergiji* (1952); *Symposium allergologicum internationale Zagrabiae-Spalati-Phari* (1964); *Peti kongres dermatovenerologa Jugoslavije*, Zagreb (1965); *Radovi Medicinskog fakulteta u Zagrebu* (1953-1958); and *Simpozij o alergozama respiratornog trakta*, Hvar (1957). He organized three national congresses of dermatology and venereology (Split, 1930; Zagreb, 1933; and Ljubljana, 1957); *I. naučni sastanak dermatovenerologa FNJR* (Zagreb, 1950); *I. kongres alergologa Jugoslavije* (1963); and *Dies italico-jugoslavici medicinae destinati* (Hvar, 1968 and 1971). He initiated Interclinical Meetings of Departments of Dermatology and Venereology Zagreb-Graz, which were alternatively held at the respective departments every 1-2 years. The main leit-motif of Kogoj's work was and remained throughout his career: *Salus aegroti suprema lex* [4,11].

He was especially engaged in the topics of allergology. He published books on prurigo, urticaria, strophulus and medicamentous exanthema [4]. He was not only interested in the clinical manifestations of allergic hypersensitivity in dermatoses but also in the biological process of sensitization [1]. Kogoj showed restraint in adopting the terms of atopy and atopic diseases, defined by Coca as hereditary susceptibility to allergic sensitization induced by atopens and provoked by atopic reagents, if the author also considered urticarial reaction as atopic allergosis. Kogoj considered eczema as allergic dermatosis and a professional disease. The rate of these disorders is relatively high in individuals at occupational exposure to antigens that can induce allergic hypersensitivity of the skin. The number of these patients is constantly increasing. The cutaneous allergic reaction is expected to disappear upon cessation of allergen exposure, which is achieved in most but not all cases. Eczema does not clear when the process has turned chronic, even if the patient's skin is not exposed to the sensitizing antigen anymore. The reason for eczema relapses has not yet been fully elucidated. Perhaps a prolonged reaction of the hypersensitive tissue is involved, and the process of hypersensitivity might in part be explained by immune induction of cellular disintegration of the unrecognized and damaged cell [1,4].

It was on Kogoj's initiative that the YASA Allergology Center was opened in 1965 in Hvar, first in the premises adapted for this specific purpose, and in 1973 moved to the newly constructed premises with the ward, clinics, diagnostic laboratories for biochemistry tests, pulmonary function testing, electrocardiography, radiology, physiotherapy, and library [1].

The major role of allergology and clinical immunology in dermatology and venereology is well known. This makes the research in the field initiated and systematically performed by Academician Franjo Kogoj at University Department of Dermatology and Venereology in Zagreb highly valuable indeed. Allergology and clinical immunology

in dermatology and venereology were his favorite fields of work and research he pursued in his rich scientific opus. He always tended to elucidate allergic manifestations on the skin or some other organ, and to clarify immune events causally related to these manifestations [4,14-52]. Kogoj began his systematic work on the issues of allergology and immunology as early as 1928, with his paper *O alergiji* (On the allergy) [14]. Since then, in his papers he used to bring some new concepts along with those widely known. Dermatoses may occasionally show similar or nearly identical symptomatology; therefore they can be differentiated and classified as allergic or non-allergic lesions only on the basis of the pathogenetic mechanism of their onset [4]. Kogoj discussed the role of many other substances and factors involved in allergic and immune reactions, such as spreading factor, histamine, acetylcholine, serotonin and peripheral nervous system [23]. Vulgar eczema was his favorite topic, addressed in many of his papers. In his monograph *Problematika egzema* (The problems of eczema, 1947), Kogoj presents the complex nature of the issue concerning manifold terms, hypotheses and theories, statements and facts. Eczema only refers to a skin manifestation where lesions involve the epidermis and occur consequentially to allergic sensitization. It develops in individuals free from hereditary allergy burden but have general predisposition for sensitization and special predisposition for eczema. All other dermatoses, no matter how similar or even clinically identical to vulgar eczema, should not be considered as vulgar eczema. Various dermatitides (e.g., *d. eczematosa*, *d. detritiva*, *d. simplex*, *d. varicosa*, *d. pediculosa*, *d. solaris*, *d. intertriginosa*) and so-called seborrheic eczema (*d. seborrheica*), by Kogoj termed "*dermatitis dyseborrheica*", are not vulgar eczema because they are underlain by sebaceous gland dysfunction rather than allergic sensitization [4,21,42,43,46]. Neither is *dermatitis eczematoides allergica* vulgar eczema, although its clinical picture may occasionally be quite identical to that of vulgar eczema, because the pathogenetic mechanism of its occurrence differs from vulgar eczema. It is in fact an eczematoid allergoid formed by hematogenous or lymphogenous dissemination of secondary eczematogen from the focus to the specifically sensitized skin [38,42]. Pruridermatitis (neurodermitis) is also an allergic dermatosis and may in some circumstances present the same clinical picture as vulgar eczema, on eczematization in particular, but it is not eczema [4,22,44]. Here, the process of sensitization takes place in connective tissue and blood vessels, and can only manifest on the genetic basis predisposing to hypersensitivity reaction [4,22,44,47]. The term "atopy" is only acceptable to denote atopic disease that manifests following previous sensitization underlain by genetic predisposition [4,44].

Kogoj bequeathed us with a rich opus of works thoroughly elaborating allergic and immune events, to mention only *Urtikarija*, *strophulus i prurigo* (Urticaria, strophulus and prurigo), published in the book *Bolesti kože* (Zagreb, 1971) and in the great Jadasohn's *Handbuch der Haut* (1962) [4,34,35]. Based on his studies, he concludes that prurigo

remains one of the most complex and inadequately clarified issues in dermatology. It is characterized by the prurigo papule located in the epidermis. Prurigo of Hebra is always an allergic dermatosis caused by some exogenous allergen. The paper *Dijagnostika alergijskih oboljenja* (The diagnosis of allergic diseases, by F. Kogoj, A. Brnobić and J. Fettich, 1962) extensively presents general indicators of allergic state and specific allergic tests used *in vivo* and *in vitro* in the diagnosis of allergic diseases (36). Results of the authors' own research into vulgar eczema, pruridermatitis, strophulus, prurigo, urticaria, and some other allergic and non-allergic dermatologic and non-dermatologic diseases are tabularly presented [4,36].

Kogoj's paper *Ein Vierteljahrhundert Vasculitis*, presented at the Deutsch-japanischer Angiologen Kongress in Essen, 1973, aroused high interest [4,52]. Kogoj used to emphasize that every problem deserves to be paid due attention and efforts irrespective of the positive or negative outcome of the results expected because the outcome will show whether the problem has or has not been properly set [53].

F. Kogoj and A. Brnobić were founders of the CMA Section of Allergology in 1952, with the intention to integrate the work of medical professions related to the study of allergic events. In 1965, the YASA Allergology Center in Hvar was founded on Kogoj's initiative. Almost all activities required in allergology and clinical immunology have been available at the Center since 1973. Kogoj published around 230 scientific and professional papers, some 70 of them dealing with the issues in allergology and immunobiology. He wrote 5 books, several monographs, and a number of precious contributions to handbooks and textbooks in the field. Upon retirement, Kogoj used to visit the Department until 1979, still working hard and having published more than 50 papers, the last one in 1977 [4,52].

Academician Kogoj received high orders, awards and decorations not only at the national level but also from other European and non-European countries for his extraordinary scientific, professional and educational work as well as for his numerous social activities [4,10,11].

Academician Kogoj departed this life but his work and achievements live. It will certainly take decades for such a bright figure, such a man, humanist, organizer, educator, health professional and researcher to appear in our environment.

References

- [1] Midžić S. Komemoracija Franji Kogoj, redovnom članu Jugoslavenske akademije znanosti i umjetnosti. Spomenica posvećena preminulom franji Kogoj, spomenica preminulim Akademcima. Vol. 28. Ikić D, ed. Zagreb: Department of Medical Sciences, Yugoslav Academy of Science and Arts, 1985.
- [2] Lipozenčić J. Katedra za dermatovenerologiju i Medicinski fakultet – jučer. Akademik Franjo Kogoj. In: Lipozenčić J, Čikeš N, Marinović B, eds. Katedra za derma-

- tovenerologiju Medicinskog fakulteta Sveučilišta u Zagrebu – 85 godina (1921.-2006.). Zagreb: Durieux, 2006; pp. 19-23.
- [3] Čećuk Lj, Belicza B, Škrbić M. Medicinski fakultet Sveučilišta u Zagrebu. Zagreb: Stvarnost, 1984.
- [4] Brnobić A, Lipozenčić J. Uloga Franje Kogoja u razvoju alergologije i kliničke imunologije u dermatovenerologiji u Hrvatskoj. Rad Hrvatske akademije znanosti i umjetnosti. Medicinske znanosti. Vol. XXVII, 466; 1994; pp. 19-26.
- [5] Kogoj F. O kauzalnom problemu u dermatovenerologiji i medicini uopće. Lijec Vjesn 1929;3:99-115.
- [6] Schwarzwald M. U povodu osamdesete obljetnice Akademika Prof. Frana Kogoja. Acta Derm Iug 1974;1:191-194.
- [7] Report by British dermatologists on their visit to University Department of Dermatology and Venereology in Zagreb 1959. Lancet 1959;??:788.
- [8] Kogoj F. *Addendum curriculum vitae*. Zagreb, 1972
- [9] Medicinski plan razvoja Dermatovenerološke klinike 1972. godine (held by A. Brnobić), 1972:32.
- [10] Brnobić A. In memoriam Akademiku prof. dr. Franjo Kogoj. Lijec Vjesn 1984;106:44-45.
- [11] Brnobić A. In memoriam Franjo Kogoj. Hautarzt 1984;35:270-271.
- [12] Kogoj F. The critical moment in syphilis treatment and times of the examination of the spinal fluid. Acta Dermatol Venereol (Stockh) 1952;32:86.
- [13] Paleček I, Čvorišćec B, Lipozenčić J. Alergologija. In: Živković R, Oberiter V, Vrhovac B. Povijest internističkih struka u Hrvatskoj. Croatian Academy of Medical Sciences, Durieux, Zagreb, 1998;95-103
- [14] Kogoj F. O alergiji. Srp Arh Celok Lek 1928;30:1-10.
- [15] Kogoj F. Über lokal verschiedene Allergie bei Pilzkrankungen. Arch Derm Syph 1928;154:421-427.
- [16] Kogoj F. O exanthemach a o jajich lokalizaciji. Cesk Dermatol 1928;9:197-200.
- [17] Kogoj F. Über die Differenzen im Grade der Allergie bei experimentellen Dermatomykosen. Arch Derm Syph 1929;158:716-723.
- [18] Kogoj F. O tuberkulinskoj reakciji s osobitim obzirom na tuberkulozu kože. Lijec Vjesn 1938;60:344-348.
- [19] Kogoj F. Hitne intervencije u dermatovenerologiji. In: Hitne intervencije praktičnog liječnika. Medicinska biblioteka. Zagreb: Naklada Liječničkog vjesnika, 1938;67/78:257-265.
- [20] Kogoj F. Liječenje medikamentoznih eritrodermija. Lijec Vjesn 1940;64:243-246.
- [21] Kogoj F. Problematika ekcema. Monografija. Zagreb: Yugoslav Academy of Science and Arts, 1947:95.

- [22] Kogoj F. Ekcem i neurodermitis. Lijec Vjesn 1948;70:169-176.
- [23] Kogoj F. Nekoliko napomena o aktualnim pitanjima o alergologiji. Poseban otisak iz "Simposion o alergiji". Zagreb: Yugoslav Academy of Science and Arts, 1952:69-77.
- [24] Kogoj F. L'épidermo-réaction superposée. Acta Allergol 1952;5:222-229.
- [25] Kogoj F. O alergiji. 10. Simposion o alergiji. Zagreb: Yugoslav Academy of Science and Arts, 1952:7-22.
- [26] Kogoj F. Alergija u dermatovenerologiji. 10. Simposion o alergiji. Zagreb: Yugoslav Academy of Science and Arts, 1952:145-158.
- [27] Kogoj F. Superponierte Tuberculin und Trichophytin-Reaction. Acta Allergol 1954;7:204-214.
- [28] Kogoj F. Dermatoses professionnelles allergiques et non allergiques. Symposium sur les dermatoses professionnelles, Lille 1955. Bull Soc Fr Dermat Syph 1955;2:210-215.
- [29] Kogoj F. La notion de la hyposensibilité en médecine. Acta Otolaryngol 1956;46:241-249.
- [30] Kogoj F. Localizzazione degli esantemi. 41th Congress SIDES, Milan, 1956. Minerva dermat. ATTI SIDES e Sez. reg. 1956;3-4:458-460.
- [31] Kogoj F. Die Therapie der Urticaria. Hautarzt 1959;10:145-147.
- [32] Kogoj F. Á propos d'une réaction au mastocyte. Acta Allergol 1959;13:349-353.
- [33] Kogoj F. Á propos d'eczéma infantile. Acta Med Jug 1959;13:268-273.
- [34] Kogoj F. Urticaria, strophulus, prurigo, pruritus. In: Kogoj F *et al.*, eds. Bolesti kože, Part 2. Zagreb: Yugoslav Academy of Science and Arts, 1971:791-814.
- [35] Kogoj F. Urticaria, strophulus, prurigo, pruritus. In: Jadassohn J, ed. Handbuch der Haut und Geschlechtskrankheiten: Ergänzungswerk IVI. Berlin: Springer Verlag, 1962:475-544.
- [36] Kogoj F. Dijagnostika alergijskih oboljenja (with Brnobić A, Fettich J). Rad Med Fak Zagrebu 1962;10:1-24.
- [37] Kogoj F. Über allgemeine Lokalisationsfaktoren bei hametogenen Exanthemen. Arch Klin Exp Dermatol 1964;219:121-126.
- [38] Kogoj F. Über allergische Ekzematoide. Hautarzt 1964;15:277-281.
- [39] Kogoj F. Gedanken zum Problem der Allergie. Symposium Allergologicum international Zagabriae-Spalati-Phari, 1964:22-34.
- [40] Kogoj F. Testversuche mit alimentären Allergenen (with Brnobić A, Luković G). Allergie Asthma 1965;11:153-156.
- [41] Kogoj F. Napomene o fiksnim egzantemima. Med Glasnik 1967;12:351-353.
- [42] Kogoj F. Kontaktekzem und Ekzematoide (with Fettich J). XIII Congress Int. Dermatol. München, 1967. Munich: Springer Verlag, 1968:244.
- [43] Kogoj F. Dijagnoza profesionalnog egzema. Lecture held at Yugoslav Academy of Science and Arts, Zagreb, 1968

- [44] *Kogoj F.* Atopičke bolesti. Simpozij o alergiji respiratornog trakta. Hvar, 1967. Zagreb: Yugoslav Academy of Science and Arts, 1968:11-16.
- [45] *Kogoj F.* Über den Einfluss wiederholter Trichophytininjektionen auf die Reaktionsfähigkeit des Testgebietes. Congress of Romanian Society of Dermatology with International Participation, 1964. *Hautarzt* 1968;19:121-124.
- [46] *Kogoj F.* Dijagnoza profesionalnog egzema. *Rad, Yugoslav Academy of Science and Arts*, 1968;38:5-29.
- [47] *Kogoj F.* Über Klimatherapie im allgemeinen und der Neurodermitis (Pruridermatitis allergica chronica constitutionalis) im besonderen. *Clinica Europea* 1969;8:139-142.
- [48] *Kogoj F.* Alergija i dermatovenerologija. In: III Kongres alergologa Jugoslavije, Sarajevo 1969, Proceedings. Sarajevo: Svjetlost, 1970:449-452.
- [49] *Kogoj F.* Uloga histamina u egzemskoj reakciji zamorčeta (with Brnobić A). In: IV Kongres alergologa Jugoslavije, Ljubljana 1972, Proceedings. Ljubljana, 1973:73-77.
- [50] La pathogenèse des *exanthema fixes*. Congrès des Dermatologues de la langue française, Proceedings. Geneva, 1973. *Toxicoderm Hig* 1974; 334-336.
- [51] *Kogoj F.* Osservazioni sulla patogenesi delle dermatopatie medicamentose (with Brnobić A). *G Min Derm* 1974;109:3-37.
- [52] *Kogoj F.* Ein Vierteljahrhundert Vasculitis. Deutsch Japanischer Angiologen Kongress, Essen 1973. *Acta Angiol* 1977;22:313.
- [53] *Kogoj F.* Interview at Radio Beograd II. 1980. Recorded on two tapes, authorized and photocopied (by the author) 1980:51. (held by A. Brnobić).

Sažetak

Akademik Franjo Kogoj i alergologija u Hrvatskoj

Franjo Kogoj bio je redoviti član JAZU u Zagrebu (1947.), prvi tajnik Razreda za medicinske znanosti Jugoslavenske akademije znanosti i umjetnosti i njen u više mandata birani potpredsjednik (1958-1973), dugogodišnji predstojnik Dermatovenerološke klinike i Katedre, više puta dekan i prodekan Medicinskog fakulteta Sveučilišta u Zagrebu. Mnogobrojni se radovi akademika Kogoja odnose na alergijska zbivanja u organizmu i na njihovo značenje za morfologiju i tijek bolesti kao i na prognozu, profilaksu i liječenje. Tu pripadaju radovi o antikutinima i prokutinima, superponirane tuberkulinske reakcije, superponirane epikutane reakcije, superponirane tuberkulinsko-trihofitinske reakcije, eksperimentalna istraživanja alergijskih reakcija kod ekcema i pruridematitisa, koji je naziv uveo u dermatološku terminologiju umjesto naziva neurodermitis odnosno endogeni ekcem, prurigo-astma, prurigo Besnier. U velikom broju radova obrađuje problematiku alergije, donoseći pri tome uvijek nešto nova. Pitanje alergije predstavlja, njegovo najomiljenije područje. U tim radovima i nadalje obrađuje svoju omiljelu problematiku: medikamentozne egzanteme, medikamentozne dermatopatije, egzem, pruridermatitis (atopijski dermatitis), pruritus, superponirane trihofitinske reakcije, upliv serotonina na alergijske kožne testove, alergijska zbivanja na koži, tzv. kortikotropinsku terapiju u dermatologiji i ulogu histamina u ekcemaškoj reakciji. Posebnu pažnju pobudila su njegova najnovija izlaganja u radu: "Ein Vierteljahrhundert Vasculitis" iznešena na "Deutsch-japanischer Angiologen Kongress" održanom u Essenu 1973. god. Njegovom je inicijativom osnovan 1965. godine Alergološki centar Jugoslavenske akademije znanosti i umjetnosti na Hvaru, koji je u početku bio smješten u prostorije adaptirane u tu svrhu, a 1973. premješten u suvremeno izgrađeni prostor za bolnički odjel, za ambulantne, dijagnostičke laboratorije za biokemijske pretrage, ispitivanje plućne i funkcije, snimanje elektrokardiograma, zatim za radiološke preglede, fizioterapiju i biblioteku. Kogoj i A. Brnobić osnivači su Alergološke sekcije Zbora liječnika Hrvatske (1952). Osnivanjem Sekcije željeli su ujediniti rad medicinskih struka koje su težile poučavanju alergijskih zbivanja. Na Kogojevu inicijativu osnovan je 1965. godine Alergološki centar Jugoslavenske akademije znanosti i umjetnosti u Hvaru. U Centru se od 1973. godine mogao neometano odvijati i gotovo sav prijeko potreban rad s područja alergologije i kliničke imunologije. Kogoj je publicirao oko 230 znanstvenih i stručnih radova, od kojih oko 70 obrađuje alergologijsku i imunobiologijsku problematiku, 5 knjiga, nekoliko monografija, a dao je i posebno vrijedne priloge za priručnike te udžbenike.

S njime je završila sjajna era povijesti Medicinskog fakulteta Sveučilišta u Zagrebu i HAZU.

Ključne riječi: Akademik Franjo Kogoj i alergologija; Pruridermatitis allergica chronica constitutionalis; Tajnik Razreda medicinskih znanosti JAZU

NOVEL CONCEPTS IN IMMUNOLOGY AND THEIR APPLICATION IN CLINICAL ALLERGOLOGY

Alenka Gagro

Institute of Immunology, Zagreb, Croatia

Summary

The objective of this review is to present some novel concepts in the field of immunology that help us understand the pathophysiological mechanisms of allergic diseases. The recognition of their heterogeneous nature in combination with animal models and sophisticated *in vitro* immunologic methods should help us translate these findings to clinical practice in order to upgrade the prevention, diagnosis and treatment of allergic diseases.

Keywords: immunology; allergology; *in vitro*

INTRODUCTION

Descriptions of the clinical presentation of allergic diseases have been known for centuries, and according to some sources as early as ancient Egypt, however, the true understanding of the diseases flared up from the beginning of the 20th century with the development of immunology and the work of von Behring, Koch and Bordet. Portier and Richet were the first to perform laboratory studies of allergy with their famous tests of immunization, sensitization and anaphylaxis. The discovery that immunization does not only protect but may also induce hypersensitivity was the cornerstone of research in allergology. With his definition of allergy as a state of altered immunoreactivity, von Pirquet directed further development of allergology as a new clinical discipline.

The current knowledge about innate and cellular immunity, role of B- and T-lymphocytes, role of the thymus, regulation of immunoglobulin genes and T-lymphocyte receptors *via* RAG1 and RAG2 enzyme activities, and the discovery that immunocytes

Correspondence to: Institute of Immunology, Rockefellerova 10, HR-10000 Zagreb, Croatia;
E-mail: agagro@imz.hr

secrete soluble factors, cytokines, that are involved in immunoreactions, has been daily enriched with ever new concepts, thus rendering the area of immunology (and allergology perceived as a field of immunology) one of the most dynamic fields of biomedicine.

Currently, more than 350 leukocyte molecules (Cluster of Differentiation, CD) are known, which enable differentiation of various types of immunocytes and 35 interleukins (IL), along with intensive development of new methods for the detection of various factors for the diagnosis and understanding of therapeutic effect variations among patients with allergic diseases.

Like in other basic biomedicine research, there is still a great need for the numerous novel concepts to extrapolate efficiently and meaningfully in daily clinical practice. The key questions of highest relevance in allergology, such as the cause of the disease onset, the pathogenesis and prevention of the disease, have only in part been answered and underlay the development of new therapeutic approaches.

DO WE KNOW WHY ALLERGIES DO OCCUR?

According to the current concept on the development of allergic diseases, these are chronic diseases that occur due to the complex interaction of the environment and individual gene expression at various ages. A great number of genes have been identified to date, whose mutations and/or polymorphisms are associated with atopy and/or allergy, and a modified anti-allergic therapy response; more than 20 have been characterized for asthma alone [1]. The hygiene hypothesis on the effect of microorganisms at an early age (or even *in utero*) to determine the individual's immunoreactivity by acting upon the mechanisms of innate immunity tries to explain the rising incidence of allergies in industrialized countries [2]. Attention has recently been focused on so-called Toll-like receptors and their ligands (e.g., lipopolysaccharide). Genetic studies have only in part confirmed this hypothesis and steered further research to a more complex area requiring additional interdisciplinary studies in the fields of genetics, immunology and epidemiology (such as the GABRIEL project that is under way in Europe since 2006), in order to detect the genes that are truly relevant for allergies, in terms of protection in particular [3,4]. Research efforts have now been focused on the genes that are specifically present in those body compartments where allergic reaction is being manifested (e.g., ADAM33 in respiratory epithelium) [5].

There is still a dilemma whether sensitization already develops *in utero*, thus the recommendation on avoiding allergens during pregnancy, at least in case of children at a high risk of developing allergy (positive family history) being scientifically questionable [6]. The reduced ability of neonatal dendritic cells to secrete IL-12, one of the main cytokines mediating Th1-immunoreaction, is considered crucial for the previously char-

acterized susceptibility of the newborns whose T-lymphocytes are more difficult to activate by the allergen, then predominantly secreting Th2-cytokines (IL-4, IL-5, IL-9, IL-13) [7]. Despite these developments, positive family history remains a major risk factor, since biomarkers of adequate sensitivity are still lacking [8].

The features that characterize an antigen as an allergen are of special importance for proper understanding of the genesis of allergies [9]. Most allergens are parts of proteins with different biologic activities (e.g., proteases, protein carriers, profilins, calcium binding proteins), and also lipids recognized by the immune system as expressed by CD1 molecules [10]. The mechanisms that lead to allergic diseases have been well understood, however, this does not hold for the molecular structures of allergens responsible for their occurrence, which makes their standardization a difficult task. Some allergens can directly influence the development of IgE-mediated immunoreactions (e.g., house dust proteolytic enzymes), whereas others favor their prolonged presence in the environment or the body (e.g., food allergens). The International Association of Immunologic Societies Committee in collaboration with the World Health Organization has been issuing and regularly supplementing the existing nomenclature of allergens (for more detail please refer to the official web site www.allergen.org). More than 40 different allergens have been successfully sequenced, thus allowing for the development of recombinant allergens for diagnostic and therapeutic purposes [11].

THE MECHANISMS OF INNATE AND ACQUIRED IMMUNITY IN ALLERGOLOGY

Along with preserved integrity of the skin and mucosa, various soluble factors and innate immunity cells make the first-line defense against all antigens including allergens.

Allergens can directly activate the complement, an important proinflammatory non-specific system, by its proteases, or indirectly *via* proteases such as trypsin, thrombin and elastase from mastocytes and other inflammatory cells. Th2-cytokines (IL-4, IL-13) also induce the secretion of C3 in epithelial cells, and a similar action has also been demonstrated for pollutants, tobacco smoke and ozone [12]. Children exposed to environmental tobacco smoke are known to have higher levels of the C3 complement component than unexposed children [13]. Anaphylatoxins (C3a and C5a) have a previously known action in anaphylaxis, and now the expression of their receptors is known to also be modulated by allergens and various pollutants. Interestingly enough, owing to studies in suitable animal models, the action of these anaphylatoxins is currently believed to be variable. So, C5a is considered important for the development of tolerance of dendritic cells in respiratory mucosa only in the conditions when allergic reaction has not yet occurred [14].

In humans, basophils are considered the main cell type responsible for early secretion of Th2-cytokines. Along with the expression of CD40 ligands, they enhance IgE synthesis and are therefore highly relevant for the initiation and maintenance of Th2-immunoreactions. Modern methods of detection of these cells in different body compartments have enabled their role in allergy to identify. The discovery that basophils are activated not only by allergen binding to the allergen-specific IgE on high-affinity IgE receptors (Fc ϵ RI) but also by some parasites, lectins and virus superantigens, with involvement of nonspecific IgE [15], is of great interest indeed.

Numerous defects in the mechanisms of innate immunity, including impaired secretion of antimicrobial peptides (surfactant proteins, catelicidin, defensin) and Toll-like receptor gene polymorphism, have been described in allergic diseases. Some of these impairments are primary, i.e. existing even before the onset of allergic disease, whereas others develop as a secondary phenomenon [16,17].

Mastocytes as central executive cells in the early stages of allergic inflammation are among the first to come in contact with the potential allergens [18]. They are found in all organs and vascularized tissues where, upon the allergen, IgE and Fc ϵ RI interaction, they release the content of mastocyte granules with preformed factors (histamine, neutral proteases, cytokines, proteoglycans) and initiate the synthesis of these and other factors (prostaglandins D, leukotrienes B4 and C4, cytokines, chemokines). The consequences of the action of these factors (increased vascular permeability and consequential tissue edema, bronchoconstriction, increased mucus production, leukocyte attraction) have been thoroughly investigated. The central role of mastocytes in these pathogenetic events has been confirmed owing to the detection of a suitable animal model (mouse with c-kit gene deletion). These models have also demonstrated that mastocytes play a role in non-allergic (e.g., autoimmune) diseases, i.e. they are involved in the pathogenesis of these diseases *via* numerous mechanisms such as modifying dendritic cell migration and their ability of T-lymphocyte activation; direct action upon T-lymphocytes and modifying their migration to target tissues; secretion of various cytokines and cell contact; possible action on regulatory T-lymphocytes and establishing tolerance)¹⁸. The true relevance of these results in allergic diseases in humans has not yet been established, especially considering the potential utilization of these concepts in the development of new drugs (c-kit inhibition, new tryptase inhibitors) [19].

Numerous studies of the role of eosinophils have resulted in some new concepts on the role of these cells in various diseases including allergies [20-22]. Currently, eosinophils are considered multifunctional leukocytes that are involved not only in the initiation but also in the modulation of innate and acquired immunity. The so-called secondary granules of eosinophils contain many factors (eosinophil peroxidase, major basic protein, eosinophilic cationic protein and eosinophilic neurotoxin) that primarily

act as cytotoxic molecules (in asthma, they act on the respiratory epithelium cells), while eosinophilic cationic protein and eosinophilic neurotoxin also act as ribonucleases. Eosinophils are activated by various stimuli (nonspecific tissue damage, infection, transplant, allergens and tumor cells). Besides preformed factors, they can also secrete cytokines, chemokines, lipid mediators and neuromodulators. In addition, eosinophils present antigens/allergens to T-lymphocytes and influence their polarization by releasing indoleamine 2,3-dioxygenase, an enzyme important for oxidative metabolism of tryptophan, from which kynurenine, an important regulator of Th1/Th2 balance, is subsequently formed [23]. The major cationic protein is also involved in the development of bronchial hyperreactivity by acting upon vagal muscarinic M2-receptors [20]. By releasing great amounts of leukotrienes, eosinophils increase vascular permeability and bronchospasm. Clinical trials have shown that pulmonary eosinophilia in asthma patients is associated with chronic coughing but not with bronchial hyperreactivity. The administration of humanized anti-IL-5 in asthma patients has only in part proved useful in blocking the major cytokine responsible for the formation of eosinophils, thus posing new questions on the role of other molecules (e.g., eotaxin) in the genesis of pulmonary eosinophilia [24,25].

Recent studies have also been focused on the subgroup of CD1-associated NK-cells expressing T-lymphocyte receptor on their surface (iNKT-cells). Studies in animals without these NK-cells have suggested that they may have a major role in shifting immunoreactions toward Th2. These cells were found in a number of patients with allergic asthma [26].

Studies initiated in the 1980s resulted in a changed opinion about differences in the allergen-specific effector helper T-lymphocytes that predominantly release Th2-interleukins (IL-4, IL-5, IL-9, IL-13) responsible for the onset and numerous manifestations of allergic disease. So, IL-10 and interferon- γ (IFN- γ) were considered as modulating cytokines enabling allergen tolerance in healthy individuals. Based on these concepts, many studies were performed measuring cytokines by various techniques and in various analytical specimens (e.g., peripheral blood, serum, skin and nasal mucosa biopsy specimens, bronchoalveolar lavage, induced sputum, etc.). However, recent studies have indicated that Th1-lymphocytes, i.e. those that predominantly release IFN- γ , can also lead to severe allergic inflammation. With the discovery of Th17, a novel subgroup of inflammatory T-lymphocytes, attempts have been made to link the previously described phenomenon of granulocyte accumulation with consequential inflammation at the site of allergic reaction (e.g., in the airways) [27]. These findings point to the need of simultaneous determination of different cytokines, primarily at the site of allergic inflammation, rather than in peripheral blood or serum samples, as mostly done to date [28].

Although B-lymphocytes have long been considered to play passive role in immunoreactions including allergies, they are not simply the cells that release IgE upon ap-

appropriate activation by the allergen, cytokines and contact with allergen-specific T-lymphocytes. Naïve B-lymphocytes can induce the formation of regulatory T-lymphocytes, which play an important role in the onset of allergies and probably also in the restitution of immunotolerance following successful hyposensitization²⁹. It has recently been demonstrated on a mouse model that IgE+ cells are mostly found beyond germinal centers that represent special compartments within the secondary lymphatic tissue, formed upon contact with the antigen. In these centers, the formation of B-lymphocytes that will release immunoglobulins or so-called memory B-lymphocytes is enabled by somatic hypermutation and maturation of immunoglobulin genes and rebinding of immunoglobulin classes. Another source of IgE-secreting plasma cells is created by the additional sequential rebinding of B-lymphocytes that release IgG1 under the action of the cytokine IL-4 (IL-21 has an opposite action). This may explain the persistence of IgE seen in allergic diseases³⁰.

It remains to be investigated whether STAT3, its mutation resulting in the occurrence of primary immunodeficiency of hyper-IgE syndrome, plays a role in allergic diseases, and if so, what its role is [31].

Regulatory T-lymphocytes are a T-lymphocyte population which is important for the establishment of peripheral immunotolerance, and are of special relevance in allergies, autoimmune disorders, tumors, infections and transplantation medicine for the control of local immunoreaction [32]. In fact, this T-lymphocyte population has a suppressor effect on other cells of the immune system, thus contributing to local modulation of the immune reaction. There are two main subpopulations of regulatory T-lymphocytes, i.e. natural regulatory T-lymphocytes as a separate line of T-lymphocytes developing in the thymus, and inducible regulatory T-lymphocytes developing in the periphery upon contact with the allergen and acting primarily *via* cytokines (IL-10 and TGF- β). The role of regulatory T-lymphocytes has also been intensively investigated in the field of allergies and asthma [33]. The ability of regulatory T-lymphocytes to suppress the allergen induced proliferation of effector T-lymphocytes during pollination season is lower in atopy patients than in healthy individuals. Similarly, they also play a major role in other forms of allergy, e.g., in contact hypersensitivity and UV activity. Furthermore, regulatory cells are involved in the development of tolerance to food allergens. Children allergic to cow's milk have a lower level and reduced functional ability of regulatory T-lymphocytes. After several-month abstinence from milk consumption, the children that had developed milk tolerance and "overcome allergy" showed elevated levels of regulatory T-lymphocytes in the circulation. Functional modulation of the existing and/or formation of new regulatory T-lymphocytes have been described as one of the mechanisms of efficient hyposensitization [34].

HOW TO PREVENT THE ONSET/EXACERBATIO OF ALLERGIC DISEASES?

By carefully designed clinical studies, efforts have been invested to reconsider the existing and develop novel procedures for the prevention of allergic diseases. As mentioned above, recommendations on avoiding allergens during pregnancy, at least in children at a high risk of developing allergies (positive family history), have been shown to be scientifically questionable [6]. Inadequate preservation of the skin and mucosa integrity contributes significantly to the occurrence of allergic diseases because the effects of air pollutants, tobacco smoke and ozone on the allergens, inflammatory mediators and cells involved in allergic reactions are at least partially known. Atopic dermatitis in early infancy begins as a disease in the pathogenesis of which IgE is not involved. A change in the skin barrier enables gradual sensitization to allergens, and then also to own antigens, so at long term the disease can be considered an autoimmune disorder as well. The phenotype and function of dendritic cells of the skin are modified by the calcineurin inhibitors, thus they have been postulated to help in establishing permanent tolerance [35].

Great attention has lately been paid to different viruses, primarily in asthma exacerbation (rhinoviruses, influenza virus), and also in the genesis of asthma (respiratory syncytial virus, metapneumovirus) [36]. The prospective studies published to date have demonstrated that bronchiolitis caused by respiratory syncytial virus can increase the risk of wheeziness, and in some studies of developing asthma later in life. However, it should be borne in mind that only a part of children develop severe infection (i.e. bronchiolitis), which is now primarily related to polymorphisms of the genes involved in nonspecific immunity [37]. Taking in consideration current trends in allergic diseases, which also point to the role of innate immunity in the development of allergen tolerance, additional studies are expected to elucidate whether a more severe picture of viral infections can in fact identify the children with predisposition to asthma, or may even lead to its development.

NOVEL APPROACHES IN THE MANAGEMENT OF ALLERGIC DISEASES

In addition to the known omalizumab, a humanized monoclonal antibody against IgE, another biological, etanercept, a soluble TNF- α receptor, has also been tested in asthma patients. The agent proved efficacious in patients with severe asthma and predominant Th1-phenotype but not in those with allergen-mediated inflammation, bronchial hyperreactivity and mild/moderate clinical picture [38].

Highly promising is DNA therapy, i.e. administration of immunostimulatory non-methylated DNA to enhance innate immunity and Th-1 immunoreaction. Also, anti-

sense oligonucleotides could be used for target blocking of respective RNA [39]. The anti-inflammatory activity of antibiotics (macrolides), chemokine antagonists (e.g., eotaxin), transcription factor antagonists (e.g., NF- κ B), and the activity of various factors on mucus production (e.g., aerosol surfactants) have been investigated [3,40,41]. Development of new respiratory virus vaccines (respiratory syncytial virus in particular) should help control the effect of viral infections in the onset and/or exacerbations of asthma⁴².

Continuing research into the immunopathogenesis of allergic diseases along with new discoveries in the fields of genomics, proteomics, pharmacogenetics and molecular pharmacology is expected to result in the advent of novel drugs, thus upgrading the control of these disorders.

References

- [1] Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. *Curr Opin Allergy Clin Immunol* 2007;7:75-82.
- [2] Vercelli D. Mechanisms of the hygiene hypothesis – molecular and otherwise. *Curr Opin Immunol* 2006;18:733-7.
- [3] Hall IP, Sayers I. Pharmacogenetics and asthma: false hope or new dawn? *Eur Respir J* 2007;29:1239-45.
- [4] Ruth L. A breath of hope. Researchers are combining their efforts to study the genetic and environmental factors in asthma. *EMBO Rep* 2007;8:319-21.
- [5] Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J* 2007;29:793-803.
- [6] Rowe J, Kusel M, Holt BJ et al. Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort. *J Allergy Clin Immunol* 2007;119:1164-73.
- [7] Prescott SL, Taylor A, King B et al. Neonatal interleukin-12 capacity is associated with variations in allergen-specific immune responses in the neonatal and postnatal periods. *Clin Exp Allergy* 2003;33:566-72.
- [8] Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy* 2003;58:1187-94.
- [9] Esch RE. Allergens. *Clin Rev Allergy Immunol* 2006;30:71-2.
- [10] Spinazzi F, Porcelli SA. Recognition of lipids from pollens by CD1-restricted T cells. *Immunol Allergy Clin North Am* 2007;27:79-92.
- [11] Chapman MD, Pomés A, Breiteneder H, Ferreira F. Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007;119:414-20.
- [12]. Köhl J, Wills-Karp M. A dual role for complement in allergic asthma. *Curr Opin Pharmacol* 2007;7:283-9.

- [13] Shima M, Adachi M. Effects of environmental tobacco smoke on serum levels of acute phase proteins in schoolchildren. *Prev Med* 1996;25:617-24.
- [14] Kohl J, Baelder R, Lewkowich IP et al. A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. *J Clin Invest* 2006;116:783-96.
- [15] Falcone FH, Zillikens D, Gibbs BF. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. *Exp Dermatol* 2006;15:855-64.
- [16] McGirt LY, Beck LA. Innate immune defects in atopic dermatitis. *J Allergy Clin Immunol* 2006;118:202-8.
- [17] Schröder NW, Maurer M. Innate immune defects in atopic dermatitis. *J Allergy Clin Immunol* 2006;118:202-8.
- [18] Gregory GD, Brown MA. Mast cells in allergy and autoimmunity: implications for adaptive immunity. *Methods Mol Biol* 2006;315:35-50.
- [19] Sayed BA, Brown MA. Mast cells as modulators of T-cell responses. *Immunol Rev* 2007;217:53-64.
- [20] Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* 2006;24:147-74.
- [21] Green RH, Brightling CE, McKenna S et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715-21.
- [22] Fortuna AM, Feixas T, González M, Casan P. Diagnostic utility of inflammatory biomarkers in asthma: exhaled nitric oxide and induced sputum eosinophil count. *Respir Med* 2007. (in press)
- [23] Odemuyiwa SO, Ghahary A, Li Y et al. Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol* 2004;173:5909-13.
- [24] Flood-Page P, Menzies-Gow A, Phipps S et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029-36.
- [25] Mattes J, Yang M, Mahalingam S et al. Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002;195:1433-44.
- [26] Meyer EH, DeKruyff RH, Umetsu DT. iNKT cells in allergic disease. *Curr Top Microbiol Immunol* 2007;314:269-91.
- [27] Bullens DM, Truyen E, Coteur L et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 2006;7:135.
- [28] Bullens DM. Measuring T cell cytokines in allergic upper and lower airway inflammation: can we move to the clinic? *Inflamm Allergy Drug Targets* 2007;6:81-90.
- [29] Reichardt P, Dornbach B, Rong S et al. Naïve B cells generate regulatory T cells in the presence of a mature immunologic synapse. *Blood* 2007;110:1519-29.

- [30] Erazo A, Kutchukhidze N, Leung M et al. Unique maturation program of the IgE response *in vivo*. *Immunity* 2007;26:191-203.
- [31] Minegishi Y, Saito M, Tsuchiya S et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007;448:1058-62.
- [32] Rouse BT. Regulatory T cells in health and disease. *J Intern Med* 2007;262:78-95.
- [33] Marinic I, Gagro A, Rabatic S. Regulatory T cells. *Acta Med Croatica* 2006;60:447-56.
- [34] Wing K, Sakaguchi S. Regulatory T cells as potential immunotherapy in allergy. *Curr Opin Allergy Clin Immunol* 2006;6:482-8.
- [35] Allam JP, Bieber T, Novak N. Recent highlights in the pathophysiology of atopic eczema. *Int Arch Allergy Immunol* 2005;136:191-7.
- [36] Papadopoulos NG, Xepapadaki P, Mallia P et al. Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA2LEN and InterAirways document. *Allergy* 2007;62:457-70.
- [37] Janssen R, Bont L, Siezen CL et al. Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes. *J Infect Dis* 2007;196:826-34.
- [38] Berry M, Brightling C, Pavord I, Wardlaw A. TNF-alpha in asthma. *Curr Opin Pharmacol* 2007;7:279-82.
- [39] Wang LC, Lee JH, Yang YH, Lin YT, Chiang BL. New biological approaches in asthma: DNA-based therapy. *Curr Med Chem* 2007;14:1607-18.
- [40] Black PN. Antibiotics for the treatment of asthma. *Curr Opin Pharmacol* 2007;7:266-71.
- [41] Rubin BK, Fink JB. Novel medications for asthma: a look at the future. *Expert Opin Invest Drugs* 2007;16:889-97.
- [42] Bueving HJ, van der Wouden JC. What is the role of virus vaccination in patients with asthma? *Curr Allergy Asthma Rep* 2007;7:72-6.

Sažetak

Suvremene spoznaje u imunologiji i njihova primjena u kliničkoj alergologiji

U ovom revijskom prikazu namjera je prikazati neke nove spoznaje iz područja imunologije koja su pomogla razumijevanju patofizioloških mehanizama alergijskih bolesti. Prepoznavanjem složene naravi tih bolesti i uporabom životinjskih modela te suvremenih imunoloških metoda istraživanja *in vitro* nastoji se iste rabiti u rutinskom kliničkom radu u svrhu uspješnije prevencije, dijagnostike i liječenja alergijskih bolesti.

Gljučne riječi: imunologija; alergologija; *in vitro*

ATOPIC DERMATITIS

Jasna Lipozenčić, Suzana Ljubojević

University Department of Dermatology and Venerology, Zagreb University Hospital
Center and School of Medicine, Zagreb, Croatia

Summary

Variable terminology used for atopic dermatitis, definition of this chronic inflammatory disease with hereditary component characterized by severe itch, typical clinical picture, and quite common association with other atopic diseases such as asthma and/or rhinitis are presented. The prevalence of this atopic disease is on a constant increase, now ranging from 0.6% and 20.5%. The course of the disease is relapsing with remissions of variable length. Remission is observed after the second or fifth year of life in one third of patients. About 40% of children with atopic dermatitis develop bronchial asthma later in life. It is considered to be caused by exposure to pollen, minor sources of infection, pets, older maternal age and numerous food allergens. Environmental factors and microbial agents (bacteria, fungi, *Malassezia furfur*, dermatophytes, viruses, etc.), psychosomatic factors, clinical picture in different age groups, other associated dermatoses, diagnostic and therapeutic procedures are described in detail.

Keywords: atopic eczema; neurodermatitis constitutionalis; Besnier's prurigo; atopic dermatitis

SYNONYMS

The various names such as atopic dermatitis (AD); atopic eczema, endogenous eczema, dermatitis atopica, neurodermitis constitutionalis, intrinsic allergic dermatitis, eczema flexurarum, Besnier's prurigo, diathetic prurigo, asthma-eczema, hay fever-eczema.

DEFINITION

AD is a chronic inflammatory skin disease due to genetic predisposition, characterized by pruritus, typical age-specific clinical picture, and frequently associated with

Correspondence to: Professor Jasna Lipozenčić, MD, PhD, University Department of Dermatology and Venerology, Zagreb University Hospital Center and School of Medicine, Šalata 4, 10000 Zagreb, Croatia; e-mail: jasna.lipozenccic@zg.htnet.hr

other atopic diseases such as asthma and/or allergic rhinitis. The prevalence of AD is rather high, mostly involving children.

Table 1. Diagnostic features of atopic dermatitis according to Hanifin and Rajka (ref. 8)

MAJOR FEATURES (must have 3 or more)

Pruritus

Typical morphology and distribution of skin lesions

- flexural lichenification and linearity in adults
- facial and extensor involvement in infants and young children

Chronic or chronically relapsing dermatitis

Personal or family history of atopy (asthma, allergic rhinoconjunctivitis, atopic dermatitis)

MINOR FEATURES (plus must have 3 or more)

Xerosis

Ichthyosis/palmar hyperlinearity/keratosis pilaris

Immediate (type I) skin test reactivity

Elevated total serum IgE

Early age of onset

Susceptibility to repeated cutaneous infection (especially *Staphylococcus aureus* and Herpes simplex and other viral infections, warts, molluscum, dermatophytes)/impaired cell-mediated immunity

Tendency toward nonspecific hand and/or foot dermatitis

Nipple eczema

Cheilitis

Recurrent conjunctivitis

Dennie-Morgan infraorbital fold

Keratoconus

Anterior subcapsular cataract

Orbital darkening

Facial pallor/erythema

Pityriasis alba

Anterior neck folds

Pruritus when sweating

Intolerance to wool and lipid solvents

Perifollicular accentuation

Food intolerance

Course influenced by environment/emotional factors

White dermographism/delayed blanch

HISTORIC ASPECTS

Atopy was adopted from the Greek word *atopos*, meaning “strange” or “unusual”. This disorder was probably first described by Robert Willan in 1808, as a prurigo-like

condition. In 1844. Hebra noted the flexural distribution of the rash, which was postulated to precede pruritus. In 1923 Coca and Cooke introduced term "atopy" to designate phenomena of hypersensitivity in human. In the 1930's Wise and Sulzberg first proposed the term "atopic dermatitis". They discussed in the 1933 *Year Book of Dermatology and Syphilology* the conditions of eczema, neurodermatitis, lichenification, and "prurigo diathésiques" described by French dermatologist Besnier and Brocq. In the follow-up studies, by Schwartz and Schnyder, first seen with infantile eczema, supported close association of bronchial asthma, hay fever, perennial rhinitis, atopic dermatitis, and some forms of food allergies with classic atopic disease. In the 1960s Ishizaka and Johanson identified a new class of immunoglobulins, the IgE antibodies, as carrier of reaginic activity, raised in atopic individuals. In the 1980's Hanifin and Rajka proposed a list of characteristic features (criteria) of AD (table 1).

EPIDEMIOLOGY

There is great worldwide variation in the prevalence of AD, ranging from 0.6 to 20.5%.

The accumulative incidence of AD before the age of 7 years has increased dramatically in recent years and the prevalence rates of AD range from 10-12% with a insignificantly greater prevalence in females compared to males (ratio 1:4). Epidemiological studies in school children show the prevalence of AD to be 15%-17%. In the last decade, the number of AD patients in Europe has been on an increase, yielding a prevalence of 20%. In 80% of patients, the onset of disease occurs before 1 year of age, and in only 2% after age 20. The course of the disease is characterized by exacerbations and remissions that cannot be etiologically explained. Complete remission has been estimated to occur in one third of patients after 2 years of age, and in another one third after 5 years of age. However, many patients with infantile AD or juvenile AD experience discomforts up to their adult age. About 40% of AD children develop bronchial asthma later in life. There are probably several reasons for the increased incidence of AD, including higher exposure to air pollution, smaller families with less exposure to infections, more pets, higher maternal age, and a wider range of foods.

Genetic aspects of atopy and atopic dermatitis

There is clearly also an important hereditary component of atopic eczema. Atopy is a condition caused by multiple gene defects. Thirty genes responsible for expression of AD have been demonstrated. Several chromosomal regions contain pathophysiologically relevant candidate genes, especially on chromosome 5q31-33. A defect on chromosome 5q31-33 in the β_2 -adrenergic gene region was found in AD patients suffering from asthma. Several studies have found the linkage of serum IgE levels to an extended region

on chromosome 5q encoding for IL-3, IL-4, IL-5, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF). There is genotypic association between the T allele of 590C/T polymorphism of the interleukin 4 gene promoter region with atopic dermatitis. This suggests that it might affect atopic dermatitis predisposition. AD has been linked to markers at chromosome 11q13, including the gene encoding for β chain of the high affinity receptor for IgE. Variants of interleukin 13 coding region, a gain-of-function polymorphism in the α subunit of the interleukin 4 receptor (16q12), and a functional mutation in the promoter region of RANTES (17q11) have a role in expression of atopic dermatitis. A gene for mast cell chymase, a key element in atopic or allergic inflammation of the skin, resides with the region on chromosome 14q11 which is thought to be specifically linked to AD.

Hanifin's group demonstrated a cyclic adenosine monophosphate (cAMP) defect with a marked increase of cAMP-phosphodiesterase in leukocytes of AD patients. Mutations of β_2 -adrenergic receptors and their reduced number on lymphocytes and keratinocytes, and impaired function of β_2 -adrenergic receptors with L-phenylalanine have been identified.

Although many genes are involved in the development of allergic diseases, particular interest is in: 1q21, 3q21 (candidate genes CD86, CD80), 5q23-31 (asthma, atopy, total IgE), 17q-25, 20p. When both parents have the same atopic disease, their child is at a 70% risk to develop the disease. The prevalence of AD in children is 56% when only one parent has AD. When parents have different atopic diseases (different phenotypes, for example: one parent has AD and other respiratory allergy), the probability for their child to develop the disease is around 30%.

PATHOGENESIS

Atopic constitution is more frequently transmitted by maternal inheritance. Various environmental factors influence clinical expression of AD, e.g., allergens, irritants, climatic and geographical conditions, body structure, psychological stress, secondary infections, etc. AD is frequently associated with immunodeficiency (selective IgA and IgM). Numerous factors are implicated in the onset of the disease. Hyperimmunoglobulinemia E is characteristic of atopic diseases, however, AD is also a consequence of immune response type IV. The cells infiltrating the skin are predominated by CD4+ Th2 cells that produce IL-4/IL-10/IL-13 and enable differentiation of B-lymphocytes and production of IgE. It has been demonstrated that Langerhans cells (LS), dendritic cells of dermis, and peripheral blood monocytes of atopic patients can bind monomeric IgE via high-affinity IgE receptors ($F_{c\epsilon}RI$), representing allergen binding molecules on antigen presenting cells. In the same way, they translate and present the allergen to T lympho-

cytes. Mastocytes also play a major role in the genesis of AD, via allergen stimulation of the secretion of mediators (histamine, prostaglandin and cytokines including interleukins IL-3, IL-4, IL-5, IL-6, and TNF- α). Thus, the function of suppressor lymphocytes (CD8) is impaired due to the underlying gene defect. In atopic individuals, T lymphocytes are mostly of Th2 type, secrete more IL-4 and IL-5, and induce IgE synthesis, IgE hyperproduction, and eosinophilia. There is a significant coordination between AD disease activity and skin eosinophilic cationic protein (ECP) deposition. AD patients have impaired metabolism of fatty acids, i.e. decreased levels of gamma-linolenic acid (GLA) and digamma-linolenic acid (DGLA). Borage (*Borago officinalis*) is a natural source of GLA, containing 24% of this acid. Inhalation allergens have a substantial role in AD. Aeroallergens penetrate the epidermis and bind to IgE expressed on Langerhans cells (LCs) via $F_{\epsilon 2}$ receptors (e.g., dermatophagoides). The pathogenesis of AD has not yet been fully clarified. The CD4+ T lymphocytes are most frequently found in the skin of AD patients. Inhalation allergens can trigger cellular response in atopic skin predominated by Th2 cells. A decreased interferon gamma (IFN- γ) secretion from activated lymphocytes and an increased interleukin 4 (IL-4) secretion are found in AD patients. Elevated level of IgE, eosinophilia, activated macrophages with an increased secretion of the granulocyte-macrophage colony-stimulating factor (GM-CSF), prostaglandin E2 (PGE2), interleukin 10 (IL-10), IL-4 and IL-5 (by Th2 cells), decreased secretion of IFN- γ (by Th1), and enhanced spontaneous basophile release of histamine have been demonstrated in atopic skin. A correlation of elevated eosinophilic protein (ECP), soluble E-selectin with eosinophilia has been reported.

The Role of Trigger in the Expression and Exacerbation of Atopic Dermatitis

Besides genetic there are other factors such as the environmental influence which consists of the climate itself, air pollution, airborne allergens (plant, animal allergens) and psychological and emotional factors which play an important role in the course of the disease.

Environmental factors

The importance of environmental factors in the development of AD has been increasing. The majority (40-65%) of AD patients experience deterioration in the winter, probably due to decreased humidity of the air outdoors (due to cold), as well as indoors due to the heating. The climate itself may influence dermo-epidermal barrier by humidity, temperature of the air and ultraviolet radiation. The aggravation by sun exposure is probably due to a non-specific intolerance of heat caused by impaired function of sweating in affected skin and induction of itch by sweating. It also differentiates the animal and plant characteristics of various geographical regions, forming specific allergenic condition. Outdoor pollution seems to be one of the major causes of the dramatic in-

crease of atopy in recent years. Chemical compounds and exhaust particles are released into the air and may have indirect effects on the allergic sensitization by favoring IgE sensitization and development of allergic disease. It has been observed that diesel exhaust particles, but not gaseous elements enhance allergy to birch pollen and nitrogen oxide and ozone have been shown to effect mucous membranes and their protective effects against penetration of allergens into the body.

Irritants

Irritants, such as soaps, detergents, disinfectants and prolonged exposure to water have and excitatory effect on the impaired barrier layer of atopic's skin. Daily washing with soap and water or noxious agent may elicit an irritant contact reaction in atopic individuals. Saliva frequently induces perioral eczema. Contact with wool is common trigger of irritant contact dermatitis in AD. Other textiles especially synthetics or dyed fabrics, are sometimes incriminated. Tobacco smoke is also potent irritator of atopic skin.

Alimentary allergens

Controlled studies have shown that nutritive allergens may lead to exacerbation of skin lesions in some AD patients. Nutritive allergens are primarily important in children. The most common allergens in children are egg-white, cow's milk, peanuts, soya, shellfish and flour. The prevalence of food allergy in AD varies widely according to different studies from 25-60%. There are also non-specific irritant reactions predominantly to acid fruit (citrus fruit, tomatoes) and salty or spicy items. Aggravation of eczema may also be provoked by food additives. The term contact urticaria syndrome (CUS) was introduced in 1975, and denotes the occurrence of urticaria and erythema as a reaction to food substances absorbed through intact skin. The IgE mediated nutritive CUS plays a significant role in AD patients. Therefore, the skin application food test (SAFT) has been developed for use in the diagnosis of this syndrome. The test has proved useful in the diagnosis of food hypersensitivity in small children with AD. However, the test sensitivity declines after the age of 4 years.

Aeroallergens

Among airborne allergens the most important are grass, weeds and tree pollen allergens, animal epidermis and dander allergens, house dust mites. Airborne allergy in AD varies widely according to different studies from 50-90%. It seems that airborne allergy is mainly IgE-mediated, and the skin lesions may be provoked by direct contact of aeroallergens with atopic skin, involving the immediate and delayed type of allergic reaction. Epicutaneous application of aeroallergens (e.g. house dust mites, weeds, animal dander, and moulds) by atopy patch test on unaffected atopic skin elicits eczematous reaction in 30-50% of the patients with atopic dermatitis.

Microbiological factors

1. Bacterial

Staphylococcus aureus

Great attention has been paid to skin colonization and infection with *Staphylococcus (S.) aureus*. This bacterium can be isolated in nostrils and intertriginous regions in 5%-15% of normal individuals, but is found in 64-100% of skin lesions in AD patients. Although the majority of patients with AD are colonized by *S. aureus*, its presence does not necessarily indicate that it acts as a pathogen. Antibiotic treatment is indicated only when there is evidence of overt clinical infection or superantigen effect is suspected. It influences the course of the disease via different mechanisms: exotoxins, enzymes, superantigen and protein A.

Impetiginization is characterized by honey-colored crusts, extensive oozing, folliculitis and pyodermal lesions indicate bacterial infection. The bacterium produces a number of toxins (enterotoxin A and B, toxic shock syndrome toxin 1), known as superantigens that activate T cells and macrophages. Children with AD develop a significantly higher proliferative response to *S. aureus* itself and its toxins, enterotoxin B in particular, as well as to the decreased production of IFN- γ and increased production of IL-4. Bacterial toxins act as superantigens aligned along MHC II and can directly stimulate massive T cell proliferation. This discovery has entailed introduction of antistaphylococcal antibiotics.

Other bacteria

Other bacteria, such as streptococcal species, may be important, but little clinical or investigative information exists to document their role

2. Fungal infections

a. *Candida albicans*

It is the most common yeast. There are no reports of increased or more severe *C. albicans* infection in AD patients. However, positive prick tests with *C. albicans* occur more frequently in patients with AD than in healthy individuals.

b. *Malassezia furfur* (*Pityrosporum ovale*)

M. furfur yeasts may be a trigger factor for AD. This organism may produce positive skin prick reactions in a higher rate (49%) in patients with AD of the head, scalp and neck region. It can be also detected in the serum (specific immunoglobulin E to *P. ovale*), and could provoke positive patch-test reaction. *M. furfur* can induce an eczematous reaction in sensitized AD patients and may trigger factor for AD.

c. Dermatophytes

Dermatophytes (eg. *Trichophyton*) are suspected of occurring more frequently in atopic patients.

Viruses

Patient with AD have a minor deficiency problem in defending against viruses. However, some viral skin infections can have dramatic course. Kaposi's herpetiform and varicelliform eruptions are caused by spread of *herpes* and *varicella* viruses. Epstein-Barr virus, parainfluenza virus, respiratory syncytial virus and cytomegalovirus infections have been reported to trigger exacerbation of AD.

Psychosomatic factors

Atopic patients often respond to stress, frustration, embarrassment, or other upsetting events with increased pruritus and scratching. Between 22-80% of patients or their parents report worsening of the skin condition due to emotional tension, nervousness of conflicts in the family, mostly via induction of itch. Children with AD have been shown to be more susceptible to stress-induced skin eruptions because of a hyporesponsive hypothalamus-pituitary-adrenal axis, which blunts the body's natural ability to produce cortisol and suppress inflammation in response to stress. The presence of AD causes anxiety and depression. Evidence that mood disturbances increase and decrease as result of fluctuation of disease severity can be found in studies demonstrating that mood symptoms correlate with AD symptoms and decrease following successful AD treatment. When the higher cortical centers are activated by stress, there is an increased secretion of substance P from adrenal glands. They serve as brain peptides that are easily released by psychosocial stress, triggering or exacerbating itching, especially in patients with AD.

Hormones

One third of young female patients reported premenstrual flare-ups of their AD. Pregnancy also was noted to have an adverse effect on 52% of pregnant patients with AD during first and second trimester, but improvement was noted in the third trimester. Hormonal analyses showed a significant reduction in serum cortisol levels compared with nonpregnant women.

CLINICAL FEATURES

Atopic dermatitis is a multifaceted disease. The clinical picture, morphology and distribution of the skin lesion, varies greatly depending on the age of patient, the ethnic group he or she belongs to, the course and duration of the disease, aggravating factors

and possible complications such as superinfection. Its manifestations ranges from very mild to severe disease.

There are three classical stages of disease-infantile, childhood, and adulthood-each of which may show acute, subacute and/or chronic skin reaction. Acute lesions are characterized by intensely pruritic, erythematous papules and vesicles over erythematous skin, erosions and serous exudate. Subacute lesions form erythematous, excoriated scaly papules, whereas chronic lesions show skin thickening with pronounced skin markings (lichenification) and nodular papules. All three stages of skin lesions may frequently be present in the same patient.

Infantile phase

In more than half of AD patients, the disease begins during infancy, and in 90% of patients the onset is before 5 years. Problems with the skin usually start around the second month.

In infancy, the characteristic lesions consist of symmetrical, dry, erythematous, scaly plaques with follicular papules on the face, mostly involving the cheeks and forehead, scalp but not the perioral region. When child starts to crawl the lesions extend to upper trunk, extensor aspect of upper and lower extremities, and dorsal aspects of the hands and feet. Diaper region is usually spared due to the moisture retention of nappies. However, diaper region may be involved by contact irritant dermatitis.

Childhood phase

As children begin to move around, the eczema becomes more localised and thickened, during the second and third year of life, the clinical picture is being modified, showing characteristic papules and plaques localized primarily in large joint flexures, especially on the neck, elbows, wrists, knees and ankles. The disease may disappear at age 2 or 3 years, however, other atopic diseases and relapses may develop. Later in childhood and adolescence, lesions involving flexural areas persist, including eyelids, hands and feet, where pustules are frequently observed. Many children develop "nummular" pattern of AD. This refers to small coin-like areas of eczema scattered over the body. The appearance of these eczematous lesions may be altered in any stage of disease due to pruritus and scratching. Then, excoriations and lichenification with hyperpigmentation and hypopigmentation are seen. Mostly, AD improves during school years and it may completely clear up by the teens, although the barrier function of the skin is never entirely normal.

Adult phase

In adults, the disease is characterized by lichenification, chronic course, thickened areas in flexures, on the neck and eyelids, with chronic facial edema (Fig. 1, 2). Some

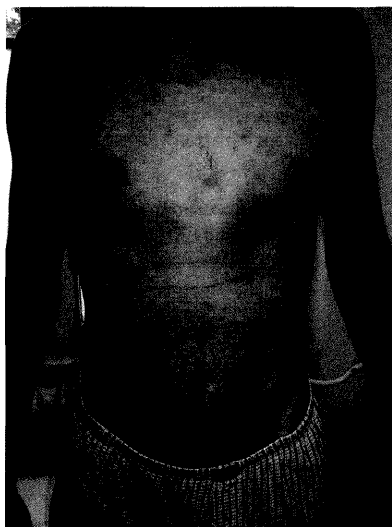


Figure 1. Adult phase, distribution

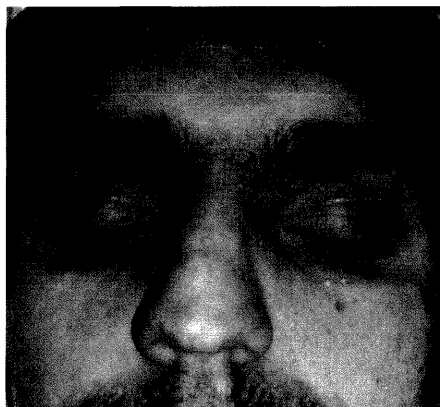


Figure 2. Erythematous facial skin, with scaliness, particularly periocular region in adult patient.

adults have prominent head and neck distribution and they manifest severe periorcular and eyelid involvement (Fig. 2). Other adults show more extremity-based disease. AD is major contributing factor to occupational irritant or allergic contact dermatitis. This often affects hands that are frequently exposed to water, detergents and/or solvents. Localized patches of AD can occur on the nipples, especially in adolescent and young women. The disease has a chronic or chronic-relapsing course, with alternating periods of regression and exacerbation of skin changes with pruritus. In some patients, a seasonal variant of the disease is present, with exacerbations mostly in spring and autumn. About 80% of patients with AD will develop allergic rhinitis, conjunctivitis or asthma.

Subtypes of atopic dermatitis

Although most patients with AD have high concentrations of total and allergen-specific serum IgE levels and positive skin prick tests reactions to common environmental allergens, a subgroup of AD patients, both children and adults suffer from skin disease with clinical features that clinically resemble the skin lesions and distribution patterns found in AD. Clinical term for such clinical features is “*pure*” type of AD and is characterized without concomitant respiratory allergies. “*Mixed*” type of AD is characterized by concomitant respiratory allergies such as asthma or rhinoconjunctivitis, polyvalent IgE sensitization against inhalants and foodstuff. Pure AD can further be subdivided into an *intrinsic* (IAD) and an *extrinsic* type (EAD). Extrinsic type affects 70-80% of patients and an intrinsic type affects 20-30% patients. In IAD is characterized by: clinical phenotype of AD, according to Hanifin and Rajka; absence of other atopic diseases such as rhinoconjunctivitis, asthma, acute urticaria, or food allergy; negative skin prick and intracutaneous tests to common aero- and food allergy; total serum IgE levels in normal ranges for infants, children, and adults; negative in vitro screening for specific IgE to common aero- and food allergens. In EAD type patients are not presented with respiratory symptoms, but IgE sensitization against inhalant and/or food allergens in the skin test or serum is present. Patients with IAD tend to have a late onset of the disease, but family history and disease duration seem to be similar in IAD and EAD. The distribution and clinical picture of the skin lesions do not differ. The classification to the two types is not just academic because in the case of IAD pharmacological prevention of asthma with long term medication is not indicated because onset of respiratory disease is quite improbable. In 2001 in an attempt to harmonize the allergy-related relevant definitions in the various fields, a task force of European Academy for Allergology and Clinical Immunology (EAACI) proposed to use as an umbrella the term *atopic eczema/dermatitis syndrome* (AEDS). AEDS is further subdivided into allergic, IgE associated group (AAEDS) and nonallergic one (NAAEDS).

Associated clinical features

Pruritus

Severe pruritus is the main characteristic of AD. The more so, pruritus with consequential scratching is an important factor for the occurrence of typical eczematoid changes in these patients. It worsens in the evening, during sweating and by wearing wool clothing. The pathogenesis of pruritus is rather complex. Histamine is not believed to be the essential mediator of itch in AD. Proteases, kinins, prostaglandins, neuropeptides, acetylcholine, cytokines, and opoids can cause itch or potentiate histamine release when injected into atopic skin.

Keratosis pilaris

Keratosis pilaris is seen most commonly during childhood and presents as small, rough, raised lesions (papules). It is regarded as a defect of keratinization at the xerotic hair follicles, in which the follicular openings are filled with horny plugs making skin feel like sand paper or chicken/turkey skin. These papules are described as "spiny and keratotic" and are typically skin colored. It occurs in 55% of atopic patients with or without AD. It is frequently noted during childhood, peaks during adolescence, and becomes less apparent during adulthood. The papules are usually found on the outer surface of the upper arms and thighs, buttocks, and face, although it may occur elsewhere on the body. The roughness is accentuated by dry skin and the condition is often worse in the winter. It tends to be inherited and may be associated with atopic dermatitis. Keratosis pilaris is asymptomatic but cosmetically disturbing.

Xerosis cutis

Xerosis cutis is considered to be the most common condition dermatosis of atopic individuals. It can persist for life, independent of the activity of atopic symptoms. The seasonal variations are noted in 75% of the patients. Due to the xerotic epidermis, skin barrier is abnormal and that provokes and sustains inflammation by activation of an epidermis-initiated cytokine cascade. The impaired barrier function of atopic skin allows great absorption of irritant agents and contact allergens. It is also easier access for bacteria, viruses, and dermatophytes.

Pityriasis alba

Pityriasis alba (PA) is a benign, chronic skin disorder that affects some children, usually between the ages 6 to 12 years, but may occur up to age 20-30. Synonyms are: pityriasis streptogenes, pityriasis simplex, pityriasis sicca facies, erythema streptogenes, furfuraceous impetigo. It is characterized with small white patches of lighter skin main-

ly on the face, although the neck, upper chest, and arms are sometimes involved. They sometimes have pinpoint-size white papules. The light colored patch seems to blend gradually into normal appearing skin, so the borders of the lesions are not clearly visible. The etiology and pathogenesis of pityriasis alba are still poorly understood. Recent studies have found direct correlation between the incidence of pityriasis alba and atopy, amount of sun exposure, lack of sunscreen use, and bathing frequency. Ultrastructurally the number of melanocytes is reduced, as are the melanosomes within both melanocytes and keratinocytes. Pityriasis alba may be interpreted as resolving atopic dermatitis in which the inflammation has subsided, thereby unveiling hypomelanosis. It is often an incidental finding on physical examination because it is usually asymptomatic. It appears to get worse when the skin is dry. It is often thought of as a mild form of eczema. During the summertime when the surrounding skin gets tanner, white patches become more visible, because it remains the same colour. In the winter months the skin is drier and the patches are more flakier. Differential diagnosis includes: pityriasis versicolor (alba), which is an autoimmune response to a fungus on the skin, and the diagnosis can be ruled out by KOH examination. Sometimes pityriasis alba can be confused with vitiligo. The vitiligo patches have very distinct border with a sharp line between normal and lighter-colored skin. Psoriatic leukoderma is rarely located on the face and the active psoriatic lesions can be found elsewhere. Treatment of pityriasis alba is not necessary since it will resolve on its own. Using moisturizer or hydrocortisone may make the patches go away faster. However, even with treatment, it may take months to years for white patches to resolve. Usage of sunscreen or protective clothing to prevent sunburn in affected areas is recommended.

Ichthyosis vulgaris

The most common disorder of keratinization with diffuse scaling and highly variable degree of involvement. The skin changes are usually not present at birth. They can be seen from early infancy to the teens. There is clear association with atopic dermatitis (25-33%). When a patient with ichthyosis vulgaris has severe pruritus and flexural involvement, usually has atopic dermatitis.

Dennie-Morgan lines

Dennie-Morgan lines are symmetrical, prominent folds, extending from medial aspect of the lower eye lid. It is most often seen in the patients with Down's syndrome, and in 60-80% of atopic individuals. These folds are usually present at birth, or appear shortly thereafter, and persist for life. Patients tend to have more than one fold, especially at the early age. The sign is present in about 70% of atopic children.

Palmoplantar hyperlinearity

People with atopic dermatitis frequently have thickening of the skin on the palms and soles with an increase number of lines in the skin (hyperlinearity). This characteristic is closely associated with genetic predisposition (atopy).

Cheilitis

Cheilitis is noted as a persistent scaliness, usually restricted to the vermilion, but often extending onto the perioral skin. It often starts in winter during childhood and appears as dry scaly lips and is often attributed to frequent licking to hydrate the dryness (cheilitis sicca). It also occurs as a result of food allergy, and in the children with no known atopy or allergy. It may become secondary infected and crusted. It can easily be transformed to a true perioral dermatitis by repeated application of potent corticosteroids.

Lichen simplex chronicus

Lichen simplex chronicus also known as circumscribed neurodermatitis or lichen Vidal, is a common pruritic skin disorder, usually sharply localized and characterized by skin thickening and prominent skin markings. Lichenification is a common phenomenon-it describes the response of the skin to repetitive irritation with thickening and more prominent skin markings. A minor itch may encourage scratching, which increases the irritation, leading to more scratching. This ultimately results in a rough, scratched (excoriated), thickened skin surface which may develop increased pigmentation (hyperpigmentation) as seen on the front part of the foot, just below the leg, nape, distal extremities, genitalia and upper eyelids. Many patients have atopic dermatitis and some have defined lichen simplex chronicus as part of the atopic diathesis.

Prurigo nodularis

Prurigo nodularis is a very pruritic dermatosis characterized by persistent nodules. In the past it was considered pathognomonic for uremia and renal transplantation patients. Probably the biggest group are patients with severe atopic dermatitis or at least an atopic diathesis. Elevated serum immunoglobulin E and increased incidence of immediate reactions to pollens, dust and house dust mites support the role of underlying atopy. Underlying emotional stress (depression, psychosocial problems) is also applied here. The lesions are almost limited to distal aspects of extremities. The face and trunk are spared. There are usually a modest number of symmetrically distributed, isolated firm nodules.

Nipple dermatitis

Nipple dermatitis is noted in 12-23% of patients with AD. It is most common in postpubertal girls. The very sensitive areolar skin Koebnerizes with the slightest rubbing or friction of clothing. It is frequently symmetrical, scaly, oozing, and papulovesicular, and it may extend onto the adjacent breast skin.

Complications of atopic dermatitis

Any chronic illness can have a major impact on the patient's life. Young children often suffer sleep disturbances which are difficult for parents and may cause behaviour problems in the child. Older children may become shy and withdrawn due to the embarrassment of a visible skin condition. Adults also suffer shyness and withdrawal due to the appearance of the eczema. Long lasting AD may lead to pronounced psychical trauma. *Retarded grow* can be associated with atopic dermatitis-it used to be seen in severe cases of AD before advent of corticosteroid therapy, and can therefore be attributed to the disease.

Because of frequent scratching and fissuring of the skin, *secondary infection* is not uncommon. The potential complications include: 1) bacterial infections (staphylococcal, streptococcal); 2) fungal infections (trichophytosis, candidiasis); 3) viral infections (warts, molluscum contagiosum, eczema herpeticum Kaposi; and 4) contact allergic dermatitis to fragrance mix, parabene, nickel, plastics components, rubber, etc.

Eczema herpeticum: Herpes simplex may produce widespread lesions in patients with eczema. Clinical picture is characterized by multiple grouped vesicles with associated fever.

Exfoliative dermatitis: Eczema may be the underlying disorder in this diffuse warm erythematous dermatitis that affects the entire body. Hospital admission is usually required.

Ocular problems: Eye complications are associated with severe AD. *Eyelid dermatitis* (Fig. 2) or *chronic blepharitis* are commonly associated with AD and may result in visual impairment from corneal scarring. *Cataracts* are more common in patients with atopic dermatitis. It occurs in up to 10% of the severe adolescent and adult cases. Atopic cataracts may resemble those induced by topical or systemic therapy. *Keratokonius* is a rare condition. It is due to degenerative changes in cornea, which is forced outwards by the intraocular pressure, to give rise to marked visual disturbances.

Atrophy or striae occur if fluorinated corticosteroids are used on the face or in skin folds. Systemic absorption of steroids may occur if large areas of skin are treated, particularly if high-potency medications and occlusion are combined.

HISTOPATHOLOGY

A biopsy is rarely used to diagnose AD, but occasionally performed to exclude other disorders. *Acute eczematous lesions* are characterized by marked epidermal intercellular oedema (spongiosis), antigen-presenting cells (eg. Langerhans cells, inflammatory dendritic epidermal cells, and macrophages) to a lesser extent in dermis. In the dermis with acute lesions, there is striking infiltration of CD4 activated T cells. *Chronic lichenified lesions* are characterized by an acantholytic epidermis with elongation of the rete ridges, parakeratosis, and only minimum spongiosis. Those chronic lesions have an increased number of IgE-bearing Langerhans cells and inflammatory dendritic epidermal cells in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate. These lesions also contain eosinophiles.

DIAGNOSTICS

Clinical Diagnostic

Elements contributing to the establishment of the diagnosis of AD are compiled from patient history, clinical finding, skin tests and laboratory investigation. Hanifin and Rajka were the first to attempt a systematic approach towards the standardization of the diagnosis of AD by proposing four major and 23 minor criteria for AD in 1980 (table 1). They argued that a diagnosis of AD could be established if three of the major and three of the minor criteria were present. The Lillehammer Criteria of 1994 are based on the idea that the distribution of the atopic dermatitis may differ in the infantile, childhood, and adult phase (table 3). *Diagnostic Lillehammer criteria are visible eczema in at least one of the regions, and at least one positive of the anamnestic of laboratory criteria, and at least three of the clinical, anamnestic, or laboratory criteria.* In addition, as fourth criteria, the skin disease should always have a duration of at least 6 weeks in the infantile phase or 3 months in the childhood and the adult phase.

Laboratory diagnostics

There are currently no single laboratory tests for the diagnosis of AD, there has been substantial progress in the past decade toward understanding the basis of the immune response in allergic diseases such as atopic dermatitis. Laboratory tests are chosen according to history data and physical examination. The aim of laboratory testing is identification of the respective allergen. In vitro tests are: 1. tests for detection of IgE antibodies: (a) radioimmunoassays for total IgE (RIST), specific IgE (RAST), (b) enzyme-linked immunosorbent assay (ELISA) for detection of IgE; 2) tests for detection of IgG antibodies: RAST and ELISA; 3) tests for lymphocyte determination; 4) tests for immunoglobulin determination: RIST; Immuno-electrophoresis is of utmost importance in the diagno-

sis of AD because low concentrations of immunoglobulins can be found in immunodeficient conditions.

Skin tests

In vivo tests in the diagnosis of immediate hypersensitivity are *prick test*, *scratch test* (*scarification test*) and *intradermal tests*. Intradermal tests are recommended only if skin prick test are negative. Despite the dilution, the allergen concentration is 100-1000-fold higher than prick testing and the risk of systemic allergic reaction is greater. Standard series with common aeroallergens and food allergens are employed. *Patch test* or *epicutaneous test* is used to determine delayed (type IV) hypersensitivity. In *Atopy patch test* aeroallergens, rarely food allergens are used for patch testing.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of AD includes most conditions listed in this chapter. Despite the long lists, in the most cases the diagnosis is easy. The table 2 lists a number of inflammatory skin diseases, immunodeficiencies, skin malignancies, genetic disorders, infectious diseases and infestations that might be considered as differential diagnosis

In infants problems includes seborrheic dermatitis, candidiasis, diaper rash, scabies, Langerhans cell histiocytosis. *In children and adults* it includes: contact dermatitis (allergic and nonallergic), seborrheic dermatitis, ichthyosis, scabies, lichen simplex chronicus, generalized mycosis, dermatitis herpetiformis, medicamentous exanthema, atopy-form dermatitis (atopic dermatitis-like dermatitis) associated with immunodeficiency syndrome.

TREATMENT

Successful management of atopic dermatitis requires a different approach involving skin care, identification and elimination of flare factors, and anti-inflammatory treatment.

General measures

In AD, the disturbed function of the skin barrier is the result of reduced ceramide concentrations and results in dry skin and enhanced transepidermal water loss. General measures of prevention and treatment of AD include avoiding those factors that have been identified as potential causes of the disease exacerbations. This implies avoidance of skin contact with wool, synthetic materials, and foods that can induce irritation, e.g., citrus fruit or tomato. Patients are advised to avoid staying in smoky areas, to reduce exposure to house dust, feather, and animal hair. Perspiration and itch increase with

heat exposure, while dry air contributes to skin dryness. Staying in mountains at 1500 m above sea level is useful. Staying at seaside, sea bathing and sun exposure usually prove beneficial in AD patients. However, skin sun protection is necessary, i.e. avoiding most intensive sun exposure (between 11.00 a.m. and 5.00 p.m.), along with application of sun protecting preparations and daily skin care. Skin care includes appropriate skin hygiene, which is of paramount importance. Patients with hypersensitivity to a nutritive allergen are recommended appropriate dietary regimen. On prescribing diets for children, it should be borne in mind that inappropriate diet may lead to malnutrition while even a minimal dietary deficit may cause changes in immune response.

It should be emphasized that hypersensitivity to food allergens is mostly diagnosed in children, whereas hypersensitivity to inhalative allergens and development of contact allergic dermatitis predominate in older patients.

Local therapy

Skin care preparation should be applied on the skin within three minutes (3-minute rule) of bathing, otherwise bathing will lead to skin drying instead of desirable skin hydration. *Bathing* is also important because the penetration of corticosteroids into the hydrated skin is best after bathing. Soaps with minimum defatting activity and a neutral pH are preferred. The use of oily baths followed by neutral preparations for skin ointment and hydration (creams, ointments and emulsions) several times a day is necessary in most AD patients. The higher the degree of skin dryness, the greater is required frequency of preparation usage. Preparations with the addition of urea, omega fatty acids, lipids, zinc and copper are used for this purpose. Local therapy for AD is tailored to the stage of disease: in acute stage, *indifferent creams*, bases (hydrophilic creams, oil/water emulsions); in subacute stage, mild *antiphlogistic creams* and *soft pastes*; and in chronic stage, water/oil ointments and tar preparations are used. Keratolytics and rehydrating ointments (salicylic acid, urea) applied by occlusive technique for 4-12 hours are used for hyperkeratoses/rhagades. *Water-alcohol compresses*, *soft zinc pastes* or *zinc oil* are applied in case of moist, macerated lesions, whereas oil baths and occasional use of corticosteroid preparations are recommended for all stages. *Local corticosteroid* preparations remain the most important agents in the management of AD patients for their antiinflammatory and antipruritic action. The potent corticosteroids should be avoided on the face, the genitalia, and the intertriginous area. With mild disease activity a small amount of topical corticosteroids two-three times weekly (monthly amounts in the mean range of 15 g in infants, 30 g in children and up to 60-90 g in adolescents and adults) achieves good maintenance. *Local antihistamines* are not recommended because of potential cutaneous sensitization. *Topical calcineurin inhibitors* are steroid-free preparation: tacrolimus and pimecrolimus. Most promising are macrolide derivatives for local appli-

cation, tacrolimus (Protopic® 0.1% and 0.03%) and pimecrolimus (ascomycin, SDZ.ASM981, Elidel®), which have an immunomodulatory effect. Their main advantage over corticosteroids is that these agents do not cause skin atrophy and telangiectasia because they do not affect collagen synthesis. They are especially useful for facial and intertriginous lesions. *Tar preparation* may have antipruritic and anti-inflammatory effects on the skin. They may be useful in reducing the potency of topical corticosteroids required in chronic maintenance of AD. Tar preparations should not be used on acutely inflamed skin, since this often results in skin irritation. *Capsaicin*, 0.025% cream acts on substance P release and has an antipruritic effect. In case of localized pyodermal lesions, local antibiotics, mostly mupirocin, is applied. The yeast *Pityrosporum ovale* is efficiently eliminated by use of a ketoconazole shampoo, or with miconazole alone or in combination with hydrocortisone.

Systemic therapy

Infectious agents

Skin infections with *Staphylococcus aureus* are rather common in AD patients, manifesting as madidations of skin lesions, pustules and crusts. Therefore, therapy with oral antibiotics, mostly erythromycin, azithromycin and cephalosporin preparations, is quite frequently justified.

Antihistamines

Systemic antihistamines are widely used in acute flares against itch. They may be helpful to decrease pruritus and permit sleep during flares. Antihistamines H1 receptor antagonists, especially those with sedative effect (diphenhydramine) are frequently prescribed for AD patients, however, their efficacy is variable. These agents (hydroxyzine, terfenadine, loratadine, astemizol, cetirizine) are efficacious in the control of reactions generated by mastocyte degranulation but not of T-lymphocyte mediated inflammatory processes. Yet, prolonged use of antihistamines (cetirizine, sodium chromglycate in combination with an antihistamine has a much better effect in AD children) has proved efficient. Tricyclic antidepressants, high-potent antagonists (doxepine, amitriptyline) are also useful. Leukotriene inhibitors have received considerable word-of-mouth testimonial support.

Systemic corticosteroids

The use of systemic corticosteroids, such as oral prednisone, may be required in the treatment of severe chronic AD. The dramatic clinical improvement that may occur with systemic corticosteroids may be also associated with an equally dramatic rebound flaring of atopic dermatitis after discontinuation of systemic corticosteroids. In children are used seldom only in acute phase.

Ultraviolet light

Natural sunlight is frequently beneficial to patients with AD. However, if the sunlight occurs in the setting of high heat and humidity, thereby triggering sweating and pruritus, it may be deleterious to patients. *Heliotherapy* (sun and sea therapy) is also useful. *UV B* or combined *UV AB* exposure is efficacious in AD patients. High daily doses of *UV A1* (340-400 nm) are even more efficacious. Photochemotherapy with oral photosensitizer methoxypsoralen (Meladinin tablets) followed by the *UV A* exposure (*PUVA therapy*) may be indicated in patients with severe, widespread AD with failure of topical steroid therapy or significant corticosteroid side-effects. Topical application of 0.3% 8-MOP ointment (*PUVA cream therapy*) could be used to treat palmo-plantar AD lesions.

Cyclosporine

Cyclosporine is a potent immunosuppressive drug that acts primary on T cells by suppressing cytokine transcription. Severe AD patients, refractory to topical corticosteroid therapy, can benefit from treatment with oral cyclosporine (5 mg/kg/per day). Discontinuation of treatment frequently results in rapid relapse of skin disease.

Interferons

INF- γ is known to suppress IgE responses and downregulate T_H2 proliferation and function. The treatment with recombinant INF- γ results in clinical improvement and decreases total circulating eosinophil counts.

Extracorporeal photopheresis

This treatment consists of passage of psoralen-treated leukocytes through an extracorporeal UVA light system. It could be used in severe, resistant to therapy AD patients.

Allergen immunotherapy

Available evidence of the effectiveness of immunotherapy with aeroallergens in the treatment of AD is mixed. Immunotherapy with aeroallergens must be proven for efficacy in the treatment of AD.

Other

Systemic immunosuppressants such as *methotrexate*, *azathioprine*, *mycophenolate mofetil* (MMF) are indicated in the management of resistant forms of AD and are behind cyclosporin in AD management. MMF odds with a better safety profile than cyclosporine or azathioprine, but controlled studies are needed to prove efficacy. *Thalidomide* (25-200

mg/day) is efficacious in resistant cases of AD, even in children. *Phosphodiesterase inhibitors* are agents intended for generalized forms of the disease.

AD develops as a consequence of complex etiology and pathogenesis. The severity and extent of skin lesions, and deviation in laboratory parameters, especially immunologic ones, may greatly differ from patient to patient. A consensus will have to be established in the near future on the new criteria defining AD, on the model of those set 25 years ago by Hanifin and Rajka. The ever growing prevalence of the disease worldwide underlines the role of prevention, timely recognition, and optimal treatment of the many patients with AD.

WHAT DO YOU NEED TO KNOW ABOUT THIS DISEASE?

- chronic, recurrent disease with intense pruritus, associated with personal or family history of atopy
 - predisposition and trigger factors are genetic, environmental factors, irritants, alimentary allergens, aeroallergens, microbial factors (*Staphylococcus aureus*, *Malassezia furfur*, *Herpes* and *varicella viruses*), psychosomatic factors and hormones
 - three classical stages of the disease: infantile, childhood and adult
 - subtypes of AD: pure (intrinsic and extrinsic) and mixed
 - associated clinical features are pruritus, keratosis pilaris, xerosis, pityriasis alba, ichthyosis vulgaris, Dennie-Morgan lines, palmoplantar hyperlinearity, cheilitis, lichen simplex chronicus, prurigo nodularis and nipple dermatitis
 - treatment involves general measures, local neutral, hydrating preparations (emollients), corticosteroids and immunosuppressive local treatment, and in severe cases phototherapy, peroral corticosteroid and other immunosuppressive agents therapy
-

Table 2. Differential diagnosis of atopic dermatitis

IMMUNODEFICIENCIES	
Wiskott-Aldrich syndrome	Ataxia-telangiectasia
DiGeorge syndrome (congenital thymic aplasia)	Bruton syndrome (X-linked hypogammaglobulinemia)
Hyper-IgE syndrome	Selective IgA deficiency
Severe combined immune deficiency	Chronic granulomatous disease
GENODERMATOSES	
Com��l-Netherton syndrome	Biotin deficiency
Phenylketonuria	Hartnup disease
Hypohidrotic ectodermal dysplasia	Acrodermatitis enteropathica
METABOLIC DISEASES	
Pyridoxine (vitamin B ₆) and niacin deficiency	Multiple carboxylase deficiency
Tyrosinemia	Essential fatty acid deficiency
Histidinemia	Zinc deficiency
IMMUNOLOGIC DISORDERS	
Dermatitis herpetiformis	Dermatomyositis
Pemphigus foliaceus	Lupus erythematosus
Graft-versus-host disease	
NEOPLASTIC DISEASES	
Cutaneous T-cell lymphoma (mycosis fungoides/S��zary syndrome)	
Langerhans cell histiocytosis	
INFECTION AND INFESTATION	
Candida (chronic mucocutaneous candidiasis)	<i>Staphylococcus aureus</i>
Neonatal mucocutaneous candidiasis	<i>Sarcoptes scabiei</i>
Impetigo	HIV-associated dermatitis
<i>Herpes simplex</i>	Congenital syphilis
DERMATITIS	
Contact (allergic or irritant)	Nummular
Seborrheic	Lichen simplex chronicus
Psoriasis	Asteatotic eczema
OTHER	
Drug eruption	
Photoallergic contact dermatitis	
Chronic actinic dermatitis	

Table 3. The Lillehammer criteria for diagnosing atopic dermatitis (adopted from ref. 27)

I. THE INFANTILE PHASE (age <2 years)

A. CLINICAL

- eczema over the face or neck
 - eczema on the trunk
 - eczema on the arms or legs (extensor or flexural sites)
 - itching or scratch effects, including lichenification or impetigo
-

B. ANAMNESTIC

- a history of relapsing course or seasonal variation
 - a history of dry skin
 - a history of itching when sweating or wool intolerance
 - a history of respiratory atopy or positive family history of atopy in first-degree relatives
-

C. LABORATORY

- elevated serum IgE or positive skin prick tests
-

D. DURATION

- duration of more than 6 weeks
-

II. THE CHILDHOOD PHASE (age 2-12 years)

A. CLINICAL

- eczema over the face or neck
 - eczema in the elbows or the kneefolds
 - eczema at the wrists or ankles
 - eczema on the hands or feet, including dermatitis sicca
 - pityriasis alba or reversed eczema above (below) elbows/knees or toilet seat dermatitis
 - itching or scratch effects, including lichenification or impetigo
-

B. ANAMNESTIC

- a history of relapsing course or seasonal variation
 - a history of dry skin
 - a history of itching when sweating or wool intolerance
 - a history of respiratory atopy or positive family history of atopy in first-degree relatives
-

C. LABORATORY

- elevated serum IgE or positive skin prick tests
-

D. DURATION

- duration of more than 3 months
-

III. THE ADULT PHASE (age >12 years)

A. CLINICAL

- eczema over the face or neck
 - eczema in the elbows or the kneefolds
 - eczema at the wrists or ankles
 - eczema on the hands or feet, including *dermatitis plantaris sicca*
 - *pityriasis alba* or nummular eczema on the arms or legs, or eczema on the upper trunk, including nipple eczema
 - itching or scratch effects, including lichenification or impetigo
-

B. ANAMNESTIC

- a history of relapsing course or seasonal variation
 - a history of dry skin
 - a history of itching when sweating or wool intolerance
 - a history of respiratory atopy of positive family history of atopy in first-degree relatives
-

C. LABORATORY

- elevated serum IgE or positive skin prick tests
-

D. DURATION

- duration of more than 3 months
-

References

- [1] *Schultz-Larsen F, Diepgen T, Svensson A.* The occurrence of atopic dermatitis in north Europe: an international questionnaire study. *J Am Acad Dermatol* 1996;34:760-4.
- [2] *Laughter D, Istvan JA, Tofte SJ, Hanifin JH.* The prevalence of atopic dermatitis in Oregon school children. *J Am Acad Dermatol* 2000;43:649-55.
- [3] *Hofer MF.* Atopic dermatitis: the first allergic step in children. *Rev Med Suisse Romande* 2000;120:263-7.
- [4] *Hanifin J, Chan CS.* Diagnosis and treatment of atopic dermatitis. *Dermatol Ther* 1996;1:9-18.
- [5] *Young RP, Sharp PA, Lynch JR et al.* Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J Med Genet* 1992;29:236-8.
- [6] *Lugović L.* Određivanje imunoloških čimbenika kao potvrda poremećene imunosti u atopijskom dermatitisu. Doctoral dissertation. Zagreb: School of Medicine, University of Zagreb, 2001.
- [7] *Murat-Sušić S.* Razina eozinofilnog kationskog proteina u serumu djece s atopijskim dermatitisom. MS thesis. Zagreb: School of Medicine, University of Zagreb, 2000; 67.
- [8] *Hanifin JM, Rajka G.* Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92:44-7.
- [9] *Lugović L, Lipozencić J.* Immunologic mechanisms in atopic dermatitis. *Acta Dermatovenerol Croat* 1996;4:21-9.

- [10] *Lugović L, Lipozencić J.* Are respiratory allergic diseases related to atopic dermatitis? *Coll Antropol* 2000;24:335-45.
- [11] *Lammintausta K, Kalimo K, Raitala R, Forsten Y.* Prognosis of atopic dermatitis. A prospective study in early adulthood. *Int J Dermatol* 1991;30:563-8.
- [12] *Ring J.* Atopy. Condition, disease or syndrome? In: Ruzicka T, Ring J, Przybilla B, eds. *Handbook of atopic eczema*. Berlin: Springer-Verlag, 1991:3-27.
- [13] *Leung DYM.* Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 1995;96:302-19.
- [14] *Leung DYM, Soter NA.* Cellular and immunologic mechanisms in atopic dermatitis. *J Am Acad Dermatol* 2001;44:51-12.
- [15] *Husar K, Lipozencić J.* Alergijske manifestacije na hranu kod djece s atopijskim dermatitisom. *Paediatr Croat* 2001;45:183-5.
- [16] *Oranje AP, van Gysel D, Mulder PGH, Dieges PH.* Food-induced contact urticaria syndrome (CUS) in atopic dermatitis: reproducibility of repeated and duplicate testing with a skin provocation test, the skin application food test (SAFT). *Contact Dermatitis* 1994;31:314-8.
- [17] *Rothe MJ, Grant-Kels JM.* Atopic dermatitis: an update. *J Am Acad Dermatol* 1996;35:1-13.
- [18] *Bunikovski R, Mielke MEA, Skarabis H, Worm M, Anagnostopoulos I, Holde G et al.* Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:814-9.
- [19] *Tofte SJ, Hanifin JM.* Current management and therapy of atopic dermatitis. *J Am Acad Dermatol* 2001;44:313-6.
- [20] *Sidbury R, Hanifin JM.* Atopic dermatitis: old, new and emerging therapies for atopic dermatitis. *Dermatol Clin* 2000;18:1-11.
- [21] *Lever R.* Infection in atopic dermatitis. *Dermatol Ther* 1996;1:32-7.
- [22] *Bekersky I, Fitzsimmons W, Tanase A et al.* Nonclinical and early clinical development of tacrolimus ointment for the treatment of atopic dermatitis. *J Am Acad Dermatol* 2001;44:S17-27.
- [23] *Williams H, Robertson C, Stewart A, Ait-Khaled N, Anabwani G, Anderson R, Asher I, Beasley R, Björkstén B, Burr M, Clayton T, Crane J, Ellwood P, Keil U, Lai C, Mallol J, Martinez F, Mitchell E, Montefort S, Pearce N, Shah J, Sibbald B, Strachan D, von Mutius E, Weiland SK.* Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. *J Allergy Clin Immunol* 1999;103:125-38.
- [24] *Wuthrich B, Schmid-Grendelmeier P.* The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ("extrinsic") and the nonallergic ("intrinsic") AEDS. *J Investig Allergol Clin Immunol* 2003;13:1-5.
- [25] *Darsow U, Laïfaoui J, Kerschenlohr K, Wollenberg A, Przybilla B, Wuthrich B, Borelli S Jr, Giusti F, Seidenari S, Drzimalla K, Simon D, Disch R, Borelli S, Devillers AC, Oranje AP,*

- De Raevé L, Hachem JP, Dangois C, Blondeel A, Song M, Breuer K, Wulf A, Werfel T, Roul S, Taieb A, Bolhaar S, Bruijnzeel-Koomen C, Bronnimann M, Braathen LR, Didier-laurent A, Andre C, Ring J. The prevalence of positive reactions in the atopy patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. *Allergy* 2004;59:1318-25.
- [26] Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wuthrich B. Epidemiology, clinical features, and immunology of the "intrinsic" (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001;56:841-9.
- [27] Schultz Larsen F, Diepgen T, Svensson A. *Clinical criteria in diagnosing AD*. The Lillerhammer criteria 1994. In: Rajka G, ed: Contribution and discussion presented at the 5th International Symposium on atopic dermatitis. May 22-25, 1994, Lillehammer, Norway. *Acta Derm Venerol* (Stockh) 1996;76:115-9.
- [28] Lin RL, Janniger CK. Pityriasis alba. *Cutis* 2005;76:21-4.
- [29] Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RE, Motala C, Ortega Martell JA, Platts-Mills TA, Ring J, Thien F, Van Cauwenberge P, Williams HC. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;113:832-6.
- [30] Lugovic L, Lipozencic J, Jakic-Razumovic J. Prominent involvement of activated Th1-subset of T-cells and increased expression of receptor for IFN-gamma on keratinocytes in atopic dermatitis acute skin lesions. *Int Arch Allergy Immunol* 2005;137:125-33.
- [31] Wüthrich B, Schmid-Grendelmeier P. The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ("extrinsic") and the nonallergic ("intrinsic") AEDS. *J Investig Allergol Clin Immunol* 2003;13(1):1-5.
- [32] Silny W, Czarnecka-Operacz M. Ethioopathogenesis of atopic dermatitis. *Int Rev Allergol Clin Immunol* 1999;5:160-2.
- [33] Jappe U, Schnuch A, Uter W. Frequency of sensitization to antimicrobials in patients with atopic eczema compared with nonatopic individuals: analysis of multicentre surveillance data, 1995-1999. *Br J Dermatol* 2003;149:87-93.
- [34] Pastar Z, Lipozencic J, Ljubojevic S. Etiopathogenesis of atopic dermatitis-an overview. *Acta Dermatovenerol Croat* 2005;13:54-62.
- [35] Bobek D, Lipozencic J, Jakic-Razumovic J, Ivankovic D, Badovinac O. The presence of CD30 on T cells in the inflammatory infiltrate of acute atopic dermatitis. *Lijec Vjesn* 2004;126:291-7.
- [36] Lipozencic J. Pimecrolimus-a safe and effective local immunomodulator in the treatment of inflammatory skin diseases. *Acta Dermatovenerol Croat* 2005;13:63-9.
- [37] Gieler U, Ehlers A, Hohler T, Burkard G. The psychosocial status of patients with endogenous eczema. A study using cluster analysis for the correlation of psychological factors with somatic findings. *Hautarzt* 1990;41:416-23.

- [38] Beltrani VS, Boguniewicz M. Atopic Dermatitis. *Dermatology Online Journal* 9(2): 1. <http://dermatology.cdlib.org/92/reviews/atopy/beltrani.html>.
- [39] Leung DY, Bieber T. Atopic dermatitis. *Lancet* 2003;361:151-60.
- [40] Hanifin JM, Paller AS, Eichenfield L, Clark RA, Korman N, Weinstein G, Caro L, Jaracz E, Rico MJ. US Tacrolimus Ointment Study Group. Efficacy and safety of tacrolimus ointment treatment for up to 4 years in patients with atopic dermatitis. *J Am Acad Dermatol* 2005;53:S186-94.
- [41] Benenson S, Zimhony O, Dahan D, Solomon M, Raveh D, Schlesinger Y, Yinnon AM. Atopic dermatitis-a risk factor for invasive *Staphylococcus aureus* infections: two cases and review. *Am J Med* 2005;118:1048-51.
- [42] Turjanmaa K. The role of atopy patch tests in the diagnosis of allergy in atopic dermatitis. *Curr Opin Allergy Clin Immunol* 2005;5:425-8.
- [43] Hawkins C. The effects of eczema on children and their families: a review. *Paediatr Nurs* 2005;17:35-9.
- [44] Williams HC. Clinical practice. Atopic dermatitis. *N Engl J Med* 2005;352:2314-24.
- [45] Herman SM, Vender RB. Antihistamines in the treatment of dermatitis. *J Cutan Med Surg* 2003;7:467-73.
- [46] Darsow U, Lübbe J, Taieb A, Seidenari S, Wollenberg A, Calza AM, Giusti F, Ring J for the European Task Force on Atopic Dermatitis. *J EADV* 2005; 19: 286-95

Sažetak

Atopijski dermatitis

U radu se iznose različito nazivlje za atopijski dermatitis, definicija ove kronične upalne bolesti s nasljednom sklonošću koju obilježava izraziti svrbež, tipična klinička slika i često udruženost s drugim atopijskim bolestima kao što astma i/ili rinitis. Ova atopijska bolest je u stalnom porastu broja oboljelih i učestalost se kreće između 0,6 – 20.5%. Tijekom bolesti je recidivirajući s različito dugim remisijama. U oko trećine bolesnika remisija je nakon 2. godine života, odnosno nakon 5. godine života. Oko 40% djece s atopijskim dermatitisom razvije kasnije u životu bronhijalnu astmu. Smatra se da je uzrok tomu izloženost peludima, manjim izvorima infekcija, kontaktu sa kućnim ljubimcima, starija životna dob majke i brojni alergeni u hrani. Detaljno se navode svi faktori okoliša kao i mikrobijelni uzroci (bakterije, gljive, *Malassezia furfur*, dermatofiti, virusi), psihosomatski faktori, klinička slika u različitim dobnim skupinama, te druge udružene dermatoze kao i dijagnostički i terapijski postupak.

Ključne riječi: atopic eczema; neurodermatitis constitutionalis; prurigo Besnier; dermatitis atopica

INCREASING INCIDENCE OF ALLERGY IN CROATIA

Asja Stipić-Marković, Branimir Čvorišćec, Branko Pevec,
Mira Radulović-Pevac

Department of Clinical Immunology, Pulmology and Rheumatology,
General Hospital «Sveti Duh», Zagreb, Croatia

Summary

Allergy in Croatia is faced with technological and civilization challenges at the time of approaching full membership to European Union. Continuous, 55 year long tradition, of successful, scientific and clinic work, several generations of organ-based specialists in allergy, is the cornerstone for the constructive integration of Croatian allergology in the Europe. The incidence of allergy is increasing, according to the epidemiological data collected from 1978 till 2004 year. Current prevalence of children asthma, allergic rhinoconjunctivitis and eczema symptoms, in the continental region is 6 %, 12.1% and 7.8%, respectively. Children prevalence in the costal region of Croatia is 8.4% , 17.50% and 3.4%, at the same order. The incidence of positive skin test to inhalant allergens in children of the continental region is 52,34%. High percentage of 51.74% subjects has skin sensitivity to three or more allergens, 29.35% to one and 18.90% to two allergens. House dust mite is the most dominant allergen, equally to most of the European countries. Second rates of sensitization were observed for grasses, followed by dog and cat epithelium, Ambrosia and tree pollens. In Croatian adults, results of prevalence of atopy markers (IgE, skin test to aeroallergens and symptoms), collected for the period of 15-years (1985-1999), showed increasing trend in elevated total IgE and atopic symptoms in male, but not in the female population. In Croatian population of adult, allergic patients, pyroglyphid mites could be considered work-related allergens for fishermen. Non-pyroglyphid mites are occupational risk factors in various rural environments of Croatia. The most common and relevant contact allergens in Croatia, are nickel sulfate, cobalt chloride and carba mix, according to the results in 3293 patients from Clinic for Dermatovenerology, Clinical Hospital Centre, Zagreb.

Keywords: Prevalence of allergic diseases; rhinitis; asthma; eczema; atopy markers; Dermatophagoides pteronyssinus

Corresponding to: Doc.dr.sc.Asja Stipić-Marković, Department of Clinical Immunology, Pulmology and Rheumatology, GH «Sveti Duh», Sveti Duh 64, 10 000 Zagreb, e-mail: astipicm@inet.hr

INTRODUCTION

Clinical allergology is discipline, whose importance, in contemporary medicine, in Croatia is rising, along with numerous tasks, resulting from continuous rise in prevalence of various clinical forms of hypersensitivity.

Rational approach to the organization of clinical allergology in Croatia include implementation of standardized diagnostic and therapeutic protocols, either transferred from international initiatives or originally created, together with recognition of advances in basic sciences and its importance for routine clinic work.

Prevention, diagnosis and treatment of allergic diseases in Croatia until nowadays, was in competence of various medical professionals, like specialists in internal medicine, pediatricians, Ear, Nose and Throat (ENT) specialists, pulmonologists and dermatologists who collaborated through clinical praxis, scientific meetings and educational activities. Although organ-based specialists will continue to play an important role in the medical care of allergic patients, the Section of Allergology and Clinical Immunology of the Union of European Medical Specialists (UEMS), recommended that, national organizations work toward establishment and maintenance a core service of care, provided by allergists with a training, based on internal medicine and/or pediatrics [1].

According to this recommendations, working group in Croatia is developing specific core curriculum, and educational and training programs for allergy specialists. Logbook for evaluation of competence and progression in the learning process, are required to qualify as an allergy specialist. Specialized centres are required, for many area of expertise, and special training should be undertaken, at institutions where appropriate training is available.

Working group is consisted of representatives of Croatian Society of Allergology and Clinical Immunology, Medical School University of Zagreb, Ministry of Health Republic of Croatia and Croatian Medical Chamber.

Accepting basic recommendations of UEMS, Croatia will ensure and maintain professional competence of allergologists, improve standards of clinical practice, and contribute to making mobility of allergy specialists, between different European countries, a real possibility.

Given the very high prevalence of allergic diseases and the different medical systems throughout the world, the World Allergy Organization, an alliance of 74 national and regional allergy societies, also created consensus document to establish educational guidelines for worldwide application, to identify and correct allergy training deficiencies, and to define appropriate training goals [2].

EPIDEMIOLOGY OF ALLERGIC DISEASES IN CROATIA

Comparing the results of the studies done from 1978 until 2002, in paediatric population, the prevalence of allergic asthma in Croatia is showing continuous rise (Table 1) [3,4,5,6]. Similar trend could be seen in studies of the prevalence of allergic rhinitis and pollinosis (Table 2) [7].

Table 1. Studies on prevalence of allergic asthma in paediatric population of Croatia

Kolbas V. i sur. (Arhiv ZMD 1979;23:351-63)	1978/79.	1,30 %
Restović-Sirotković M. (Paediatr Croat 1993;37:75-81)	1982/83.	2,80 %
Banac S. (Paediatr Croat 1994;38:7-13)	1988/89.	5,90 %
Aberle N. i sur. (Paediatr Croat 1998;42:9-14)	1990.	3,60 %
Stipić-Marković A. i sur. (Acta Med Croatica 2003;57:281-5)	2000/01.	6,02 %
Banac S. i sur. (Croat Med J 2004;45:721-726)	2001/02.	8,40 %

Table 2. Studies on prevalence of allergic rhinitis and pollinosis in paediatric population of Croatia

Author	Rhinitis	Pollinosis
Restović-Sirotković M. (Paediatr Croat 1993;37:75-81)	2,94%	1,37%
Kavurić-Hafner C. i sur. (Pediater Croat 1996;40:29-34)		1,17%
Stipić-Marković A. i sur. (Acta Med Croatica 2003;57:281-5)	12,13%	7,55%
Banac S. i sur. (Croat Med J, 2004;45:721-726)	17,50%	6,70%

Two research centers (Department of Clinical Immunology, Pulmology and Rheumatology, General Hospital "Sveti Duh", Zagreb and Department of Pediatrics, Clinical Hospital Center, Rijeka), where the most recent epidemiological studies were done, used standardized methodology, developed by the International Study of Asthma and Allergies in Childhood (ISAAC) [8,9]. Due to that, Croatian results on 12-month prevalence of asthma, allergic rhinitis and atopic eczema symptoms, are comparable to the

results obtained in 155 centers worldwide [10,11]. The results showed marked difference in the prevalence of these disorders, between continental and coastal regions, being low in continental and moderate in the coastal region.

In 10 year old children, 12-month prevalence, of asthma symptoms was 6.02%, allergic rhinitis symptoms 12.13%, and atopic eczema 7.83% in the "continental study" (Figure 1).

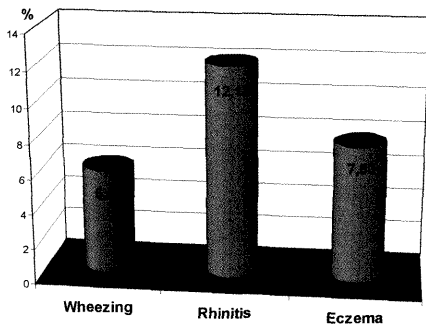


Figure 1. 12-month prevalence rates of symptoms of asthma, rhinoconjunctivitis and eczema, in 10 year old, Zagreb school children (Stipić-Marković A, Pevec B, Radulović-Pevec M, Acta Med Croat, 2004)

Estimated 12-month prevalence rates, of symptoms, in 6-7 year old children, from the coastal region of Croatia were: wheezing 9.7%, allergic rhinitis symptoms 16.9%, allergic rhinoconjunctivitis symptoms 5.6% and atopic dermatitis symptoms 5.4% (Figure 2).

In the 13-14 age group, from the coastal region, 12-month prevalences were: wheezing 8.4%, allergic rhinitis symptoms 17.5%, allergic rhinoconjunctivitis symptoms 6.7%, and atopic dermatitis symptoms 3.4% (Figure 3).

Prevalence rates of allergic symptoms were not similar with positive skin test response rates to inhalant allergens, in children population of the "continental study". The percentage of 52,34% positive skin prick test in 384 children, was much higher results than the prevalence of any of symptoms registered [12]. 29.35% of positive skin reactors were sensitized to only one of ten used allergens, 18.90% to two allergens, and a high percentage of 51.74% children with skin sensitivity were sensitized to three or

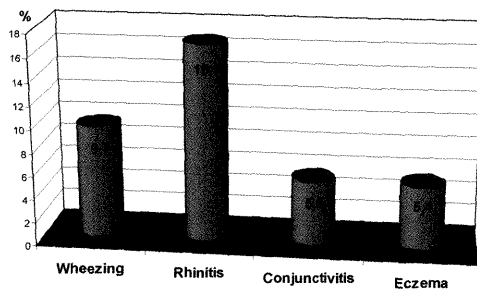


Figure 2. Estimated 12-month prevalence rates, of symptoms, in 6-7 year old children, from the coastal region of Croatia (Banac S, Lah Tomulic K, Ahel V, et al., Croat Med J, 2004)

more allergens. The most common allergen was *Dermatophagoides pteronyssinus* (56.2%), followed by grass pollen (40.3%) and *Dermatophagoides farinae* (39.8%). In addition, a high prevalence of positive SPTs to dog and cat, *Ambrosia trifida*, and tree pollen were found, while cockroach and moulds were causing sensitization in fewer number of subjects (Figure 4). Prevalence rate of sensitization to cockroach is the first report for Croatian children population, what is similar to some Mediterranean countries (13).

Although population in this study was not representative of the general children population it nevertheless, may reflect that prevalence of allergic sensitization to one or

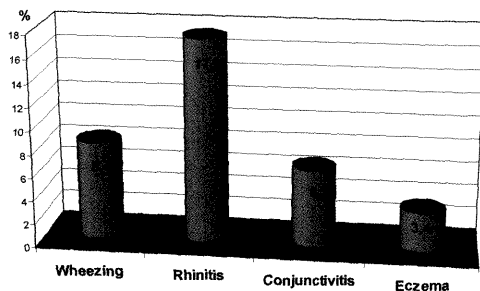


Figure 3. Estimated 12-month prevalence rates, of symptoms, in the 13-14 age group, from the coastal region of Croatia (Banac S, Lah Tomulic K, Ahel V, et al., Croat Med J, 2004)

more inhalant allergens was high. Similar prevalence of sensitization was reported amongst American school children [14].

According to a literature data, some living environmental factors are associated with an increased incidence of allergic diseases. However, the impact of environmental factors and allergic diseases is not so clear. Until recently, genetic and environmental factors have generally been studied independently. To increase the probability of identifying causative factors for complex diseases, it is necessary to study the genes and the environment to determine their interactions.

Regarding relationship between environmental factors and allergic diseases in Croatia, one investigation showed some correlations, similar with the observation of the authors from other countries [15]. Allergic and non-allergic groups of children showed statistically significant differences in attendance of kindergarten, vaccination against pertussis, pertussis infection and parasite infestation. Higher prevalence of allergy positively correlated with attendance of kindergarten without previous attendance of child care facility, correlated with male sex and with previous pertussis infection. Lower prevalence of allergy was found in children with previous parasite infestation and children who received pertussis vaccine. Exposure to tobacco smoke and damp environment with moulds and fungi were identify as risk factor for allergy in Croatia.

Prevalence of adult patients sensitivity to inhalant allergens on the Adriatic coast, was determined by the group of authors from Split [16]. In the group of 3500 patients with symptoms of rhinitis or asthma, prevalence rate was 34,2% for *Dermatophagoides pteronyssinus*, 30,7% for pollens (grass, trees, P.officinals or P.tobira), 8,5 % for animal hair, and 2,5% for molds.

Research by the same group of authors indicated that in coastal region of Croatia, *Ambrosia elatior* is an important cause of seasonal allergic rhinitis and asthma. They suggested that this allergen extract should be included in the routine diagnostic procedures in southern Croatia [17].

Exposure to *Ambrosia elatior* is important cause of allergic symptoms in Osijek-Baranja County too. In the last few years an increase in the number of people hypersensitive to ragweed has been observed. It correlated with the increase of air pollen concentration measured by use of Burkard volumetric apparatus and expressed as counts of pollen grains in mm² of air collected [18].

Authors from Institute for Medical Research and Occupational Health, Zagreb for more than fifteen years are investigating epidemiology of allergic diseases and monitoring exposure to indoor allergens in rural and urban, coastal and continental regions of Croatia.

The most recent epidemiologic study from this group of authors evaluated a 15-year trend in the prevalence of atopy markers (elevated total IgE, positive skin prick test to

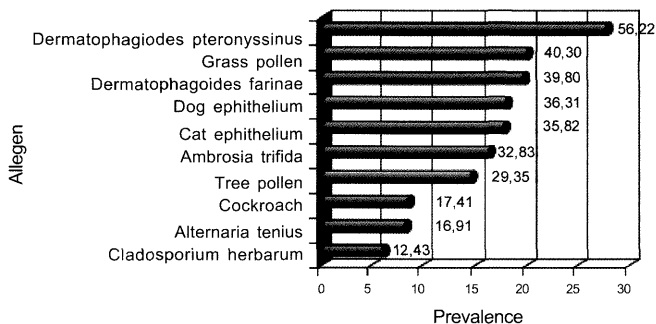


Figure 4. Distribution of different allergens inducing skin reactivity, in 10-year old, Zagreb school children, who were positive in skin prick test (n=201)

common aeroallergens and positive atopic symptoms) in Croatian adults, separately for women and men. The study included 721 subjects (445 men and 276 women), 18-45 years old, examined for allergies within pre-employment preventive examination. Results showed the increasing trend in the prevalence of concurrently elevated total IgE and positive atopic symptoms in Croatian adult male population between 1985 and 1999 (OR 2.44, 95% CI 1.39-4.29, $p=0.002$), but not in the female population. Women showed an increased prevalence of positive SPT only, with borderline significance. In women, rural residence was found as the predictor of elevated total IgE (OR 5.36, 95% CI 2.41-11.93, $p=0.000$) and smoking as the predictor of concurrently elevated total IgE and positive SPT (OR 6.20, 95% CI 1.67-23.07, $p=0.006$). [19].

Difference between the mite fauna in the inland and coastal Croatia was evaluated analyzing floor house dust samples collected from the coastal, inland rural and inland urban area. More than 70% of mites identified in all areas were pyroglyphid. Non-pyroglyphid mites participated with 20-25% of all identified mites in the coastal areas (*Blomia*, *Lepidoglyphus* and *Glycyphagus*) and with 15% in the inland areas (*Lepidoglyphus* and *Acarus*). Significantly the highest Der p 1 median levels were found in the coastal area. The highest Der f 1 median levels were found in the inland urban area, with significantly lower levels in the inland rural and coastal areas. The levels of Der f 1 were significantly higher in samples taken from households with central heating than from those with traditional heating. Compared to traditional heating, central heating significantly increases the risk of exposure to Der f 1 levels >2 mcg/g of dust (odds ratio, 7.35;

95% confidence interval, 1.43- 37.87; $P=0.01$). The results encourage the implementation of separate diagnostic dust mite lists for the coastal and inland areas due to climatic and dwelling differences [20].

Exposure to dust mites was also estimated in various occupational environments in Croatia. In total, 29 occupational dust samples were collected: 10 from urban areas (offices, archive of an insurance company, tobacco, paper-recycling, fish-processing and textile plants, animal unit for experimental rats, winery), 9 from rural areas (barley, hay, animal food and flour warehouses, tailor's shops, wood-processing plant, swine confinement house, grocer's storeroom), and 10 samples from cabins of five fishing boats (five floor and five bed samples). Microscopy showed no mites in urban areas. Pyroglyphid mites (*D. pteronyssinus*) were found in all bed samples from fishing boats. Non-pyroglyphid mites were found in samples from barley, hay and animal food warehouses, the swine confinement house, grocer's storeroom, and fishing-boats. Pyroglyphid mite allergens were detected in eight of 10 dust samples from the fishing-boats. Median levels of Der p 1, Der f 1, and Der 2 in cabin bed samples were $10\mu\text{g/g}$, $0.2\mu\text{g/g}$, and $3.5\mu\text{g/g}$, respectively. Findings on fishing boats suggest that pyroglyphid mites could be considered work-related allergens for fishermen. The results of this study confirmed non-pyroglyphid mites as occupational risk factors in various rural environments [21].

The accuracy of common criterion for positive skin prick test (SPT) reaction to dust mites (the mean wheal diameter above or equal than 3 mm) was evaluated with respect to specific IgE values of above 0.35 kUA/L (+sIgE). Specific IgE and standard SPT to *Dermatophagoides pteronyssinus* (DP) and *farinae*, *Lepidoglyphus destructor* (LD) and *Tyrophagus putrescentiae* (TP) were performed in a random sample of 457 subjects, of whom 273 men and 184 women. Where the mean wheal diameter of equal or above 3mm was considered positive (+SPT), the correlation between +SPT and +sIgE was 0.47 for DP ($p<0.001$), 0.43 for DF ($p=0.004$), 0.35 for LD ($p=0.03$) and 0.37 for TP ($p=0.014$). Regarding +sIgE, this SPT criterion has a specificity of 92.2% for DP, 82.3% for DF, 80.8% for LD and 70.1% for TP. When the value 4.5 mm was taken as the threshold for the positive SPT reaction to TP, specificity increased significantly from 70.1% to 86.4%. It was concluded that the 3mm SPT threshold criterion is not reliable in evaluating sensitisation to TP due to an insufficient specificity of the allergen extract to this mite [22].

German cockroach (*Blattella germanica*) allergen levels in house dust and skin reactivity were evaluated in adult atopic and non-atopic subjects from inland areas of Croatia. *Blattella germanica* group 2 allergen was measured in 94 house dust samples collected from living room and bedroom floors. Skin prick testing with common inhalant allergens, *Blattella germanica*, storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* and total IgE measurement were performed in 187 adult outpatients, 131 from urban and 56 from rural areas. Positive skin prick test to cockroach was observed in 9.6%.

subjects. The frequency of subjects positive to cockroach was higher in atopic than in non-atopic subjects, but not significantly (12.2% vs.4.7%). Positive skin test to storage mites was the only factor which significantly increased the risk of skin positivity to cockroach. *Blattella germanica* allergen was not detectable in house dust samples. These results suggest that there is no relevant exposure to cockroach allergen in house dust samples from inland areas of Croatia. Positive skin prick test to cockroach in atopics is in the majority of cases related to positive skin prick test to storage mites, probably due to cross-reactivity [23].

For evaluation of hypersensitivity to different contact allergens in Croatia, patch test is performing by the standard technique proposed by the International Contact Dermatitis Research Group. The most common and relevant allergens are nickel sulfate, cobalt chloride and carba mix according to the results of the authors from Zagreb Dermatovenerology Clinic. In the group of 3293 patients with allergic contact dermatitis, contact irritant dermatitis, atopic dermatitis, psoriasis vulgaris, seborrheic dermatitis and other inflammatory dermatoses results showed statistically significant differences in patch test response according to sex and age for three allergens (cobalt chloride, nickel sulfate and thiomersal decreased with age); according to occupation for nine allergens (cobalt chloride, nickel sulfate, balsam of Peru, fragrance mix, thiuram mix, wood tars, neomycin sulfate, thiomersal and detergents), and clinical diagnosis for two allergens (nickel sulfate, and wood tars). Nickel sulfate, cobalt chloride and carba mix were found in all examinees regardless of age, sex, occupation and diagnoses [24].

CONCLUSION

The data presented in this article reflect current trends in the prevalence of allergic diseases in Croatia in crucial years of negotiations and EU accession. That could serve for the strategy of development of basic and clinical allergology.

References

- [1] Malling H, Gayraud J, Papageorgiu-Saxoni, Hornung B, Rosado-Pinto J, Del Giacco SG. Objectives of training and specialty training core curriculum in allergology and clinical immunology. *Allergy* 2004;59:579-588
- [2] Kaliner MA, Del Giacco S, Crisci CD, Frew J, Liu G, Maspero J, Moon HB et al. Requirements for physician training in allergy. *Current Allergy Clin Immunol* 2006;19:113-117
- [3] Kolbas V, Lokar R, Stanic M, Krznaric-Sucic Z. Prevalence of asthma in Zagreb school children [in Croatian]. *Arhiv za zastitu majke i djeteta*, 1979;23:351-363
- [4] Restovic-Sirotkovic M. Presentation of 12 years work of the allergology-pulmonary division of the schools' polyclinic in Split [in Croatian]. *Paediatrica Croatica* 1993;37:75-81

- [5] Banac S. Prevalence of allergic childhood asthma on the islands of Cres-Losinj. *Paediatrica Croatica*, 1994;38:7-13
- [6] Aberle N, Reiner-Banovac Z. Epidemiological examination of asthma in children [in Croatian]. *Paediatrica Croatica*, 1998;42:9-14
- [7] Kavurić-Hafner C, Matika Šetić A. Features of hay fever in Istrian Children. *Paediatrica Croatica* 1996;40:29-34
- [8] Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. *Eur Respir J*, 1995;8:483-491
- [9] The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet*, 1998;351:1225-1232
- [10] Stipić-Marković A, Pevec B, Radulović-Pevac M, Custović A. Prevalence of asthma, allergic rhinitis and conjunctivitis, and atopic eczema symptoms: ISAAC in school population of Zagreb, Croatia. *Acta Med Croatica* 2003;57:281-285
- [11] Banac S, Lah Tomulic K, Ahel V, Rozmanic V, Simundic N, Zubovic S et al. Prevalence of asthma and allergic diseases in Croatian children is increasing: survey study. *Croat Med J*, 2004;45:721-726
- [12] Stipić-Marković A, Pevec B, Radulović-Pevac M, Turkalj M, Zimic L, Čvorišćec B. Skin reactivity to inhalant allergens in Zagreb school children. *Period Biol*, 2007;109 (2):161-164
- [13] Heinzerling L, Frew A.J, Bindslev-Jensen C, Bonini S et al. Standard skin prick testing and sensitization to inhalant allergens across Europe-a survey from the GA2LEN network. *Allergy* 2005;60:287-1300
- [14] Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: Results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005;116:377-383
- [15] Stipić-Marković A, Pevec B, Radulović-Pevac M, Čustović A, Predović J. Allergic diseases in relationship with environmental factors in a population of school children in Zagreb, Croatia. *Arh Hig Rada Toksikol* 2004;55:221-228
- [16] Cvitanović S, Znaor Lj, Perišić D, Grbic D. Hypersensitivity to pollen allergens on the Adriatic coast. *Arh Hig Rada Toksikol*, 2004;55:147-154
- [17] Cvitanović S, Znaor Lj, Kanceljak-Macan B, Macan J, Gudelj I, Grbic D. Allergic Rhinitis and Asthma in in Southern Croatia: Impact of Sensitization to Ambrosia elatior, *Croatian Medical Journal* 2007;48:68-75
- [18] Prus V, Čuljak Z. Preosjetljivost na pelud Ambrozije na području Osiječko-Baranjske županije. *Arh Hig Rada Toksikol* 2004;55:155-158
- [19] Macan J, Varnai VM, Maloča I, Kanceljak-Macan B. Increasing trend in atopy markers prevalence in Croatian adult population between 1985-1999. *Clin Exp Allergy* 2007; 37(12):1756

- [20] Macan J, Kanceljak B, Plavec D, Milković-Kraus S. Differences in mite fauna between the continental and Mediterranean climates of Croatia: microscopy and Dustscreen test findings. *Allergy* 2003;58 (8):780-783
- [21] Macan J, Kanceljak-Macan B, Mustać M, Milković-Kraus S. Analysis of dust samples from urban and rural occupational environments in Croatia. *Arh Hig Rada Toksikol* 2005;56(4):327-332
- [22] Kanceljak-Macan B, Macan J, Plavec D, Klepac T, Milković-Kraus S. The 3mm s skin prick test (SPT) threshold criterion is not reliable for *T. putrescentiae*: the reevaluation of SPT criterion to dust mites. *Allergy* 2002;57(12):1187-1190
- [23] Macan J, Plavec D, Kanceljak B, Milković-Kraus S. Exposure levels and skin reactivity to German cockroach (*Blattella germanica*) in Croatia. *Croat Med J* 2003;44(6):756-760
- [24] Marinović-Kulisić S, Lipozencic, J, Pastar Z. Contact allergy in sociodemography feature. XXVI Congress of the European Academy of Allergology and Clinical Immunology. *Allergy*, 2007;394 (ISBN: 0105-4538).

Sažetak

Povećana incidencija alergija u Hrvatskoj

Suvremena alergološka znanost i praksa u Republici Hrvatskoj nalazi se pred izazovima koje donosi civilizacijski i tehnološki razvoj a jedan od vidljivih znakova tih izazova je predstojeći ulazak države Hrvatske u zajednicu Europskih naroda. Zahvaljujući dugom, 55-godišnjem znanstvenom i stručnom radu alergologa iz svih specijalističkih područja medicine, hrvatska alergologija integrirana će se u Europu kao njen ravnopravni dio. Radna grupa za izradu plana i programa specijalizacije iz alergologije i kliničke imunologije izradila je prijedlog koji je u potpunosti usklađen sa preporukama Udruženja medicinskih specijalnosti Europe. Važnost suvremene kliničke alergologije u Hrvatskoj proizlazi iz zadataka koje joj nameće trend porasta prevalencije svih oblika bolesti preosjetljivosti. Prema rezultatima epidemioloških studija učinjenih po međunarodno dogovorenoj metodologiji prevalencija simptoma astme, alergijskog rinitisa i ekcema u populaciji školske djece kontinentalnog dijela zemlje iznosi redom 6%, 12.1% i 7.8%.

Prevalencije u primorskoj Hrvatskoj dio iznose 8,4%, 17,5% i 3,4%, istim redom. Prevalencija pozitivnog kožnog testa u «kontinentalnoj» studiji je 52,3%. Zapažen je visoki postotak kožne reaktivnosti na tri i više alergena (51,74%). Dominantni alergeni su grinje kućne prašine a slijede ih alergeni trava, epitel mačke i psa, korov Ambrozije te poleni stabala.

U populaciji odraslih, rezultati ispitivanja trenda prevalencije markera atopije u 15-godišnjem razdoblju (1985-1999), pokazuju uzlaznu krivulju razine IgE i simptoma alergijskih bolesti, za muškarce ali ne i za žene. Mjerenje koncentracije grinja u uzorcima prašine, dokazane i razlike u vrstama grinja, kontinentalnog i priobalnog područja, što upućuje na potrebu različitog odabira alergena za testiranje u pojedinim regijama.

Specifično za našu alergologiju je, dokaz da se piroglifidne grinje mogu smatrati profesionalnim alergenom u ribara, a nepiroglifidne grinje, profesionalni rizični čimbenik u ruralnim područjima Hrvatske.

Najčešći kontaktni alergeni našeg područja su nikal sulfat, kobalt klorid i mješavina mirisa, prema rezultatima istraživanja provedenih na 3293 pacijenta Klinike za dermatovenerologiju Kliničkog bolničkog centra Zagreb.

Ključne riječi: Prevalencija alergijskih bolesti; rinitis; astma; ekcem; markeri atopije; Dermatophagoides pteronyssinus

CURRENT LABORATORY DIAGNOSIS OF ALLERGY

Slavica Dodig

Srebrnjak Children's Hospital, Reference Center for Clinical Pediatric Allergology of
the Ministry of Health and Social Welfare, Zagreb, Croatia

Summary

The requests for laboratory diagnosis of allergic diseases refer to a battery of tests that should provide the following: 1) determine the type of allergic reaction by detecting humoral (total IgE) and cellular (eosinophilic and basophilic granulocyte count, eosinophil cationic protein) mediators of allergic reaction; 2) identify triggers of allergic reaction (specific IgE to particular causative allergens, tests for basophilic granulocyte *ex vivo* activation, leukotrienes, CD63, histamine); 3) assess the clinical course of allergic reaction (tryptase for immediate reaction, eosinophil cationic protein for delayed reaction); 4) enable specific immunotherapy monitoring (total and specific IgE, specific IgG4 and IgG1, IgG4/IgG1 ratio, proallergic and proinflammatory cytokines); and 5) estimate diagnostic efficiency of determination of particular humoral or cellular mediators of allergic reaction (i.e., sensitivity, specificity, positive and negative predictive value).

Although laboratory investigations are a useful tool in the diagnosis and management of allergic diseases many problems remain unsolved. Those problems can be grouped into three categories: preanalytical (specimen collection), analytical and post-analytical (interpretation, follow-up, retesting) phases of laboratory testing.

Keywords: allergy; IgE; basophils; leukotriene; immunotherapy

The requests made to clinical biochemists for laboratory diagnosis of allergic diseases refer to a battery of tests that should provide the following: 1) determine the type of allergic reaction by detecting humoral and cellular mediators of allergic reaction; 2) identify triggers of allergic reaction; 3) assess the clinical course of allergic reaction (immediate, delayed, or prolonged); 4) enable specific immunotherapy monitoring;

Correspondence to: Doc. dr. sc. Slavica Dodig, Ph. D., Srebrnjak Children Hospital, Srebrnjak 100, 10000 Zagreb, E-mail: slavica@dodig.net

and 5) estimate diagnostic efficiency of determination of particular humoral or cellular mediators of allergic reaction. The tests currently used and their diagnostic value in the detection and therapeutic monitoring of allergic diseases are briefly described.

1. IDENTIFICATION OF THE TYPE OF ALLERGIC REACTION

Identification of the type of allergic reaction implies determination of humoral and cellular allergic reaction mediators, including total immunoglobulin E (IgE) concentration, eosinophilic and basophilic granulocyte count [1], eosinophil cationic protein (ECP) concentration [2], identification of membrane markers of basophil activation (CD63), and histamine release from basophilic granulocytes [3].

1.1. Determination of total IgE concentration

As a simple and automated method, determination of total IgE concentration in serum is included in the screening work-up for atopy [4]. Serum concentration of IgE antibodies varies from very low levels in infancy through several-fold levels from the age of nine years (Table 1). None of other immunoglobulins undergoes such an increase within the normal range during lifetime.

Table 1. Upper limits of IgE concentration in 95% of children according to age

Age (years)	IgE, 95 th centile (kIU/L)
<1	20.2
1	30.4
2	38.6
3	57.0
4	57.2
5	65.4
6	73.0
7	82.6
8	76.4
9	98.4
10	102.6
11	104.8
12	101.4
13	97.4
14	94.2
15	105.2
16	100.0

The upper reference limits of total IgE concentration in children are consistent with cut-off values between atopic ($n=4520$) and nonatopic ($n=3355$) subjects. The values were determined at Department of Clinical Laboratory Diagnosis, Srebrnjak Children's Hospital in Zagreb (blood sampling was performed during the 2001-2004 period) [5].

In addition to its role in detecting atopic disease, determination of IgE concentration appears to point to the disease severity, in children below 16 years of age in particular [6,7]; however, some authors would not agree with the latter [8,9].

1.2. Eosinophilic and basophilic granulocyte count

Although numerous cell types (lymphocytes, eosinophilic granulocytes, mastocytes, basophilic granulocytes, endothelial cells, epithelial cells of the respiratory system, neutrophilic granulocytes, antigen-presenting cells) are involved in allergic reaction, eosinophilic granulocyte count in the blood and nasal swab is mostly determined in daily routine. The advent of blood counters offering automated determination of basophilic granulocyte count has enabled the use of this adjunct parameter in the assessment of allergic disease.

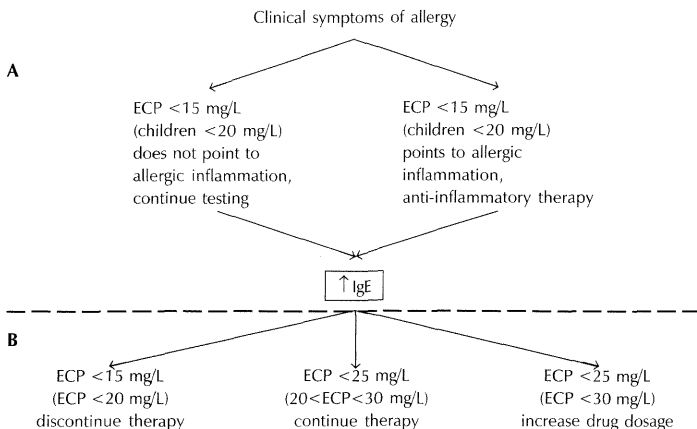


Fig. 1. Assessment of ECP value in monitoring/treatment of allergic inflammation.

1. 3. Eosinophil cationic protein (ECP) concentration

In daily routine, determination of the concentration of ECP released from eosinophilic granulocyte granules allows for the course of allergic reaction to monitor, since an increased ECP concentration (like eosinophilic granulocyte count) points to acute allergen exposure and thus to the severity of allergic inflammation [10,11]. Thus, it can be used as a marker in therapeutic monitoring in asthmatic patients. The upper reference limit is 15 ig/L for adults and 20 ig/L for children, and levels exceeding these cut-off values point to allergic inflammation (Fig. 1).

A - Increased serum ECP concentration ($> 15\mu\text{g/L}$ in adults and $> 20\mu\text{g/L}$ in children, respectively) provides an additional information to complement the clinical evaluation of allergic inflammation. B - Since therapy with corticosteroids can reduce allergic inflammation, ECP measurement can therefore be used for guiding the anti-inflammatory response. The advice is to check ECP before considering changing the treatment dose. Corticosteroid treatment may be reduced if the patient's clinical situation is stable and the ECP level is normal.

2. DETECTION OF THE TRIGGER OF ALLERGIC REACTION

Blood concentration of specific IgE to particular causative allergens detected by cutaneous testing is determined to identify the trigger of allergic reaction. In early childhood when cutaneous tests are less reliable, determination of IgE to a group of causative allergens (inhalant or nutritional; Phadiatop – Yes or No test for IgE, 93% sensitivity and 89% specificity) is recommended in screening for causative allergens, as an alternative to cutaneous testing [12]. If hypersensitivity is not demonstrated by this procedure, no further testing is required (99% negative predictive value); however, when hypersensitivity to a group of allergens is proven (89% positive predictive value), the procedure should be followed by determination of particular specific IgE in the same serum sample. It should be noted that the values obtained by Phadiatop method are expressed in arbitrary units set by the manufacturer.

2. 1. Determination of allergen-specific IgE concentrations

Upon detection of causative allergens by the skin prick test, the presence of specific IgE against these allergens and their main epitopes is determined in serum of patients with a clinical picture of allergic disease. Determination of specific IgE in serum indicates current immune activity, while skin testing reveals IgE local bioactivity mediated by long-living cells [13]. If the concentration of specific IgE is determined by the standardized method [14] (according to the World Health Organization Second International

Table 2. Serum levels of specific IgE antibodies (CAP procedure) [15]

Specific IgE (kU _A /L)	Class	Result interpretation
<0.35	0	Unmeasurable concentration of specific IgE
0.36-0.7	1	Lowest concentration of specific IgE
0.71-3.5	2	Low concentration of specific IgE
3.6-17.5	3	Moderate concentration of specific IgE
17.6-50.0	4	High concentration of specific IgE
51.0-100.0	5	Very high concentration of specific IgE
>100.0	6	Extremely high concentration of specific IgE

al Reference Preparation for Human IgE, WHO 2nd IRP 75/502), results can be quantitatively expressed within a range from 0.36 to 100 kU_A/L, except for IgE classes 1-6 (Table 2).

Table 3 shows correlation of the results obtained by determination of total and specific IgE in serum and can be used a guide in interpretation of results.

2. 2. Activation of basophilic granulocytes

This group of tests also includes determination of the concentrations of released histamine (radioimmunology, ELISA), leukotrienes (ELISA) [16,17] and surface CD markers (flow cytometry) following basophilic granulocyte activation by the causative aller-

Table 3. Interpretation of *in vitro* procedures

Total IgE (kIU/L)		Specific IgE (kIU _A /L)						
		<0.35	0.36-0.7	0.71-3.5	3.6-17.5	17.6-50.0	51.0-100.0	>100.0
Very low	No atopy		Perhaps not significant; requires repeat testing*	Possible individual hyper-sensitivity; repeat testing*	Possible individual hyper-sensitivity	Possible individual hyper-sensitivity	Possible multiple hyper-sensitivity	Possible multiple hyper-sensitivity
Normal	No atopy		Perhaps not significant; requires repeat testing*	Cut-off value; repeat testing*	Possible individual hyper-sensitivity	Possible individual hyper-sensitivity	Possible multiple hyper-sensitivity	Probably multiple hyper-sensitivity
Increased	Atopy not excluded; expand specific IgE spectrum	Atopy not excluded; expand specific IgE spectrum	Atopy not excluded; expand specific IgE spectrum	Probably multiple hyper-sensitivity history	Multiple hyper-sensitivity	Probably multiple hyper-sensitivity	Probably multiple hyper-sensitivity	Probably multiple hyper-sensitivity
Very increased	↓	↓	↓	↓	↓	↓	↓	↓

*these findings may be due to the patient's pollen hypersensitivity, therefore repeat testing upon completion of pollination may prove useful

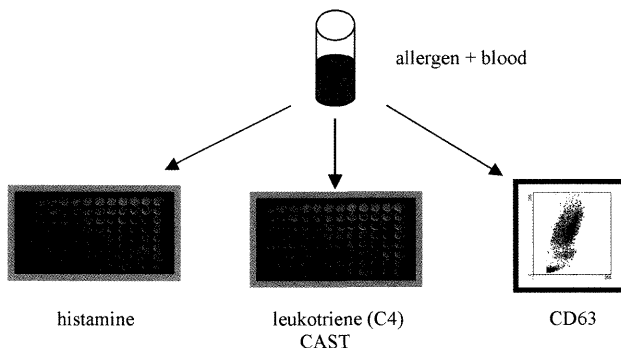


Fig. 2. The principle of methods of basophilic granulocyte activation. Basophilic granulocytes derived from patient blood are activated by the causative allergen *ex vivo*, then various markers of their activation can be determined (CAST = Cellular Allergen Stimulation Test).

gen *ex vivo* (Fig. 2) [18]. Cellular *in vitro* diagnosis has greatly improved with the introduction of the method of flow cytometry. In allergic diseases, the diagnosis based on flow cytometry is for the time being mostly employed in research studies [19]. The role of tests for basophilic granulocyte activation in the diagnosis of allergies remains to be determined.

3. ASSESSMENT OF THE CLINICAL COURSE OF ALLERGIC REACTION

Evaluation of the clinical course of allergic reaction, i.e. immediate, delayed or prolonged hypersensitivity, can theoretically be done by determination of the existing mediators such as tryptase (a marker of anaphylaxis, immediate reaction) or ECP (delayed reaction), or by determination of synthesized mediators of allergic reaction such as leukotrienes following basophilic granulocyte exposure to causative allergens. For the time being, these tests are only performed in research. Tryptase is a serine protease that is released, along with histamine, from mastocytes upon the allergen-IgE complex formation, and represents a marker of anaphylactic reaction [20]. As histamine concentration rises within 15-30 minutes and returns to normal values within one hour, it is quite difficult to measure. Therefore, the measurement of tryptase is used as a more reliable laboratory parameter. Increased tryptase concentration ($> 13.5 \text{ ng/L}$) can be demonstrated within 3-6 hours of anaphylactic reaction, then it returns to cut-off values in the next 12-14 hours.

4. SPECIFIC IMMUNOTHERAPY MONITORING

Specific immunotherapy (SIT) *in vitro* monitoring is burdened with a number of problems, from those related to the patient himself and the immune pathomechanisms that have led to the development of allergic disease; coadministration of symptomatic and anti-inflammatory therapy; long-term SIT (currently, no parameters are available to predict with certainty in the early stage of disease whether therapy will eventually prove useful for the patient); choice of biomarkers and defining their cut-off values above or below which there is or there is no protective action of an immune system segment; through laboratory procedures that undergo rapid modifications and advances, thus the results obtained at the beginning of some longitudinal studies cannot be reliably compared with the results recorded at the end of the same studies or with the results reported from other studies; in addition, many of the laboratory procedures that could be used to determine some key biomarkers (e.g., immunoglobulin subclasses, cell subpopulations, cytokines, etc.) cannot be employed in daily routine but remain reserved for scientific research. Besides clinical symptoms, some studies also monitored results of *in vivo* (cutaneous testing) and *in vitro* tests [21,22], e.g., determination of the concentration of total IgE and specific IgE [23], specific IgG [24,25], specific IgG4 and IgG1 (IgG4/IgG1 ratio) [26], IgG2, lymphocyte T subpopulation [27], proallergic cytokines (IL-4, IL-13 and IL-5), proinflammatory cytokines (IFN- γ , IL-2 and IL-12) [28], and leukotrienes [29]. It should not be forgotten that monitoring of the severity of clinical symptoms in each individual patient is of paramount importance in the overall immunotherapy monitoring in patients with allergic diseases.

Lately, recombinant allergens have been used worldwide in the diagnosis and in SIT, enabling determination of patient profile and use of individualized SIT, i.e. only those allergen determinants (epitopes) to which hypersensitivity has developed in the respective patient. Assessment of specific IgE and specific IgG4 concentrations during immunotherapy yields better results with the use of recombinant allergens than with the usual allergen preparations [30].

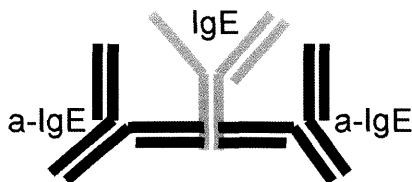


Fig. 3. Anti-IgE (a-IgE) binds free IgE.

The administration of monoclonal anti-IgE (omalizumab) results in free IgE blocking, thus reducing the level of free IgE capable of binding to mastocytes and basophilic granulocytes (Fig. 3) [31]. Therefore, determination of total IgE concentration on the very first day of omalizumab administration is useful to monitor free IgE concentration.

5. ASSESSMENT OF DIAGNOSTIC EFFICIENCY

Diagnostic procedures are expected to be accurate, precise, sensitive, specific, reliable, reproducible, and with minimal interfering factors [32,33]. A prerequisite for the most efficient use of diagnostic tests is to know their limitations, their analytical, preanalytical and postanalytical interferences, and their diagnostic efficiency (i.e. sensitivity, specificity, positive and negative predictive value, table 4). Each healthcare institution should determine diagnostic value of every diagnostic procedure *in vivo* or *in vitro*.

Table 4. Diagnostic efficiency of total and specific IgE (including Phadiatop) and ECP determination (adapted from 34, 35)

Analyte	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Total IgE	97.1	93.3	94.5	96.6
Phadiatop - adults	70.8	90.7	72.6	89.9
Phadiatop - infants	65.0	100.0	100.0	93.0
Specific IgE, asthmatic children (<i>Dermatophagoides pteronyssinus</i>)	100.0	96.7	97.3	100.0
ECP, asthmatic children	97.4	100.0	100.0	96.8

6. PROBLEMS IN DIAGNOSIS OF ALLERGY

Although laboratory investigations are a useful tool in the diagnosis and management of allergic diseases many problems remain unsolved. Those problems can be grouped into three categories: preanalytical (specimen collection), analytical (testing) and post-analytical (interpretation, follow-up, retesting) phases of laboratory testing.

6.1. Preanalytical problems

- Time of blood collection for determination of total IgE, specific IgE, ECP and eosinophil count, respectively, could be important in cases of hypersensitivity to:
 - seasonal allergens (level of selected analytes is greater in pollen season),
 - insect sting allergens (waning of allergen-specific IgE with time following exposure), and

- drug allergens (long time elapsed between the administration of the drug and specific IgE determination will result with negative specific IgE).
- The eosinophil release of ECP *in vitro* during clotting is time- and temperature-dependent. ECP levels in serum increase with time as the ECP release from the eosinophils will continue until serum is separated from the cells. Therefore the blood collection tube, coagulation time and temperature must be kept within specified limits during clotting.
- Increased concentration of total tryptase activity can be detected up to three to six hours after the anaphylactic reaction. It returns to normal value within 12-14 hours after release. Samples should preferably be collected between 15 minutes and 3 hours after the suspected event causing mast cell activation [20].

6. 2. Analytical problems

Total and specific IgE antibody determination in serum can produce different results depending on the method. Therefore standardized methods should be used.

6. 3. Post-analytical problems

Correlation of *in vitro* testing with *in vivo* testing as well as with clinical finding is the most important for diagnosis and monitoring of allergic disease [8,15]. Some problems can be specialized:

- Under ideal conditions, specific IgE determination should provide results concordant with skin prick testing. However, common allergen extracts are not used in these two methods.
- In spite of the fact that reference intervals for total IgE are defined, 5 % of non-atopic subjects have increased total IgE concentration, and up to 10% of atopic subjects might have IgE concentration inside the reference intervals [5].
- Sensitisation alone does not indicate clinically significant hypersensitivity. Conversely, a negative result does not necessarily exclude clinically significant allergy.
- No single type of *in vitro* test should be used as the only diagnostic test for food allergy.
- No biomarker can assess clinical course of allergic disease.
- Although effective SIT is accompanied by increase of allergen specific IgG/IgG4 antibodies and decrease of total/specific IgE antibodies [24-26], as yet, no early marker can predict the final outcome of SIT.

In conclusion, it should be noted that clinical biochemistry laboratories in Croatia included in the Croatian Institute of Health Insurance system perform most diagnostic procedures in the field of allergology (e.g., eosinophilic granulocyte count, total and specific IgE concentration, ECP, tryptase) by use of standardized methods, thus making

the values obtained at different laboratories comparable. In addition, these clinical biochemistry laboratories are included in international performance quality assessment, e.g., Quality Club (Pharmacia Diagnostics), thus receiving reports on performance quality in each individual laboratory for particular analytes on a monthly basis.

References

- [1] *Prussin C, Metsalfe DD.* IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2006;117:S450-6.
- [2] *Nja F, Røksund OD, Carlsen K-H.* Eosinophil cationic protein (ECP) in schoolchildren living in a mountainous area of Norway: a population-based study of ECP as a tool for diagnosing asthma in children with reference values. *Allergy* 2001;56:138-43.
- [3] *Szegedi A, Irinyi B, Gal M, Hunyadi J, Danko K, Kiss E, Sipka S, Szegedi G, Gyimesi E.* Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria. *Br J Dermatol* 2006;155:67-75.
- [4] *Platts-Mills TAE.* The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 2001;164:1-5.
- [5] *Dodig S, Richter D, Benko B, Živčić J, Raos M, Nogalo B, Čepelak I, Dodig M.* Cut-off values of total serum IgE between nonatopic and atopic children in north-west Croatia. *Clin Chem Lab Med* 2006;44:639-47.
- [6] *Siroux V, Oryszczyn MP, Paty E, Kauffman F, Pison C, Vervloet D, Pin I.* Relationships of allergic sensitization, total immunoglobulin E and blood eosinophils to asthma severity in children of the EGEA Study. *Clin Exp Allergy* 2003;33:746-51.
- [7] *Kovač K, Dodig S, Tješić-Drinković D, Raos M.* Correlation between asthma severity and serum IgE in asthmatic children sensitized to *Dermatophagoides pteronyssinus*. *Arch Med Res* 2007;38:99-105.
- [8] *Duane LS, Stein R, Halonen M, Holberg CJ, Wright A, Martinez FD.* Total serum IgE and its association with asthma symptoms and allergic sensitization among children. *J Allergy Clin Immunol* 1999;104:28-36.
- [9] *Laske N, Niggemann B.* Does the severity of atopic dermatitis correlate with serum IgE levels? *Pediatr Allergy Immunol* 2004;15:86-8.
- [10] *Prehn A, Seger RA, Faber J, Torresani T, Molinari L, Gerber A, Senhauser FH.* The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma. *Pediatr Allergy Immunol* 1998;9:197-203.
- [11] *Venge P.* Serum measurements of eosinophil cationic protein (ECP) in bronchial asthma. *Clin Exp Allergy* 1993;23(Suppl 2):3-7.
- [12] *Ballardini N, Nilsson C, Nilsson M, Lilja G.* ImmunoCAP Phadiatop Infant – a new blood test for detecting IgE sensitisation in children at 2 years of age. *Allergy* 2006;61:337-43.

- [13] Ahlstedt S. Understanding the usefulness of specific IgE tests in allergy. *Clin Exp Allergy* 2002;32:11-6.
- [14] Johansson SGO. ImmunoCAP. Specific IgE test: an objective tool for research and routine allergy diagnosis. *Expert Rev Mol Diagn* 2004;4:273-9.
- [15] Plebani M. Clinical evaluation and cost-benefit analysis of a new system (Pharmacia CAP system) for the identification of specific IgE. Uppsala: Pharmacia Diagnostics AB, 52-5103-40/01, 1991.
- [16] de Weck AL, Sanz ML. Cellular Allergen Stimulation Test (CAST) 2003, a review. *J Invest Allergol Clin Immunol* 2004;14:253-73.
- [17] Rudolf M, Milavec-Puretić V, Lipozenčić J, Malenica B. Clinical usefulness of cellular antigen stimulation test in detection of aspirin allergy. *Acta Dermatovenol Croat* 2005;13:93-6.
- [18] Demoly P, Lebel B, Arnoux B. Allergy review series X: Progress in diagnosis of allergy *in vitro* – allergen induced mediator release tests. *Allergy* 2003;58:553-8.
- [19] Dekaris D. *In vitro* dijagnostika celularnih imunoreakcija u alergijskim bolestima dišnog sustava. *Medicus* 1997;6:45-53.
- [20] Edston E, van Hage-Hamsten M, Johansson SG. Tryptase – at last a useful diagnostic marker for anaphylactic death. *Allergy* 1996;51:443-4.
- [21] Bousquet J, Calvayrac P, Guerin B et al. Immunotherapy with standardized *Dermatophagoides pteronyssinus* extract. I. *In vivo* and *in vitro* parameters after a short course of treatment. *J Allergy Clin Immunol* 1985;76:734-44.
- [22] Cramer R. Allergy diagnosis, allergen repertoires, and their implications for allergen-specific immunotherapy. *Immunol Allergy Clin North Am* 2006;26:179-89.
- [23] Raos M, Dodig S, Koncul I, Treščec A. Praćenje učinkovitosti imunoterapije astme u djece u *in vivo* i *in vitro* testu. *Paediatr Croat* 1993;37:71-3.
- [24] Einarsson R, Dreborg S, Hammarstrom L, Lofkvist T, Smith SI, Svensson G. Monitoring of mite *Dermatophagoides farinae* allergen-specific IgG and IgG subclass distribution in patients on immunotherapy. *Allergy* 1992;47:76-82.
- [25] Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2007;119:780-91.
- [26] Gehlhar K, Schlaak M, Becker WM, Bufe A. Monitoring allergen immunotherapy of pollen-allergic specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 1999;29:497-506.
- [27] Benjaponpitak S, Oro A, Maguire P, Marinkovich V, De Kruffy RH, Umetsu DT. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999;103:468-75.
- [28] Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, Staple SQ, Aalberse RC, Till SJ, Durham SR. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172:3252-9.

- [29] Kopp MV, Mayatepek E, Engels E, Brauburger J, Riedinger F, Ihorst G, Wahn U, Kuehr J. Urinary leukotriene E4 levels in children with allergic rhinitis treated with specific immunotherapy and anti-IgE (Omalizumab). *Pediatr Allergy Immunol* 2003;14:401-4.
- [30] Mothes N, Valenta R, Spitzauer S. Allergy testing: the role of recombinant allergens. *Clin Chem Lab Med* 2006;44:125-32.
- [31] Beeh KM, Beier J, Buhl R. Saisonale Schwankungen des Serum-IgE-Spiegels und deren mögliche Bedeutung für die Berechnung der therapeutischen Dosis von Omalizumab (rhuMab-E25, anti-IgE). *Pneumologie* 2004;58:546-51.
- [32] Williams PB, Barnes JH, Szeinbach SL, Sullivan TJ. Analytic precision and accuracy of commercial immunoassays for specific IgE: establishing a standard. *J Allergy Clin Immunol* 2000;105(6 Pt 1):1221-30.
- [33] Dolen WK. IgE antibody in the serum – detection and diagnostic significance. *Allergy* 2003;58:717-23.
- [34] Vidal C, Gude F, Boquete O, Fernandez-Merino MC, Meijide LM, Rey J, Lojo S, Gonzales-Quintela A. Evaluation of the Phadiatop test in the diagnosis of allergic sensitization in a general adult population. *J Invest Allergol Clin Immunol* 2005;15:124-30.
- [35] Nogalo B. Ispitivanje dijagnostičke osjetljivosti, specifičnosti i djelotvornosti testova *in vivo* i *in vitro* u djece preosjetljive na inhalacijske alergene. MS thesis. Zagreb: Zagreb University School of Medicine, 2005.

Sažetak

Suvremena laboratorijska dijagnostika alergije

Zahtjevi laboratorijske dijagnostike alergijskih bolesti odnose se na odabir analiza koje bi trebale: 1. odrediti vrstu alergijske reakcije otkrivanjem humoralnih (ukupni IgE) i staničnih (broj eozinofilnih odnosno bazofilnih granulocita, eozinofilni kationski protein); 2. otkriti pokretače alergijske reakcije (specifični IgE na pojedinačne uzročne alergene, testovi *ex vivo* aktivacije bazofilnih granulocita – leukotrijeni, CD63, histamin); 3. procijeniti klinički tijek reakcije (triptaza za ranu reakciju, eozinofilni kationski protein za kasnu reakciju); 4. omogućiti praćenje uspješnosti specifične imunoterapije (ukupni i specifični IgE, specifični IgG4 i IgG1, omjer IgG4/IgG1, proalergijski i proupalni citokini); 5. odrediti dijagnostičku djelotvornost određivanja pojedinih humoralnih ili staničnih posrednika alergijske reakcije (osjetljivost, specifičnost, pozitivna i negativna, predvidljiva vrijednost).

Iako su laboratorijska istraživanja korisna u dijagnozi i liječenju alergijskih bolesti, brojni problemi još su neriješeni. Ti se problemi mogu svrstati u tri glavne kategorije: preanalitička (uzorkovanje), analitička i poslijeanalitička (tumačenje, praćenje, ponavljno testiranje) faza laboratorijskog određivanja.

Gljučne riječi: alergija; IgE; bazofilni granulociti; leukotrijeni; imunoterapija

BOOK REVIEWS

Holubar K, Fatović-Ferenčić S, Plewig G.

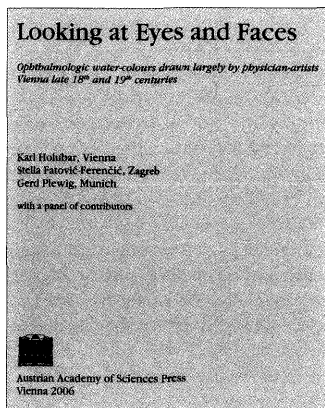


Fig. 1. Cover page

The intention of the book was, to present hand made illustrations from up to 200 years ago and have various medical specialists, ophthalmologists, dermatologists, give their immediate impressions. The authors wanted to emphasize how the eye, the hand, and the brain of a talented physician can prove dexterity and talent in several fields. A seminal figure certainly is George Joseph Beer (1763- 1821) known for founding the first university department of ophthalmology located in the Vi-

enna General Hospital in 1812. Beer was very popular at the time and many of well known showed up as his patients and acquaintances because he was an eminent eye surgeon, clinician, and a painter.

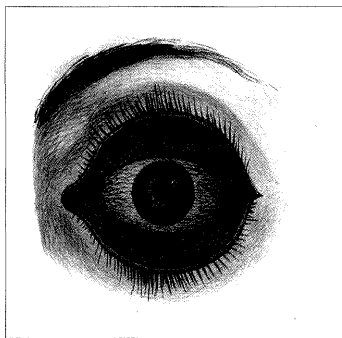


Fig. 2. An unknown author one of the pictures from the book presenting vernal conjunctivitis or atopic keratoconjunctivitis

The book contains eight chapters : *Introduction* by Walter B Shelley, *A Look Back - into the Beer-Jaeger Family* by Jan Odorich Abel (from the Beer progeny); *The Vienna School of Ophthalmology and its Roots* by Karl Holubar and Stella Fatović-Ferenčić; *The water-colours and drawings from 1782 on, clinical and histopathologic: an individual evaluation by ophthalmologists* by Edward Jaeger (Wills Eye Institute, Philadelphia and namesake of the famous Vienna ophthalmologist in the 19th century), Robert Nussenblatt (head of the National Eye Institute at the NIH in Bethesda, Günther Grabner, head of the eye department at Salzburg University, Hans Slezak (emeritus from the University of Vienna), Anselm Kampik, from the University of Munich, *Descriptions by other medical specialists; Medical Terminology, Then and Today* by Darrell Wilkinson, nestor of British dermatology, *Vision and fiction: The Eye and Visual Perception as a Metaphor in Literature* by Marita Amm, from Halle University, Germany, *Senses and Their Medialization* by Michael Metzeltin, professor of Romance languages, University of Vienna; *The Psychology of Vision: Beauty in the Brain of the Observer* by Ingo Rentschler, again a professor from the Ludwig-Maximilain University of Munich; *True to Nature: Ethics of Medical Imagery* by Stella Fatović-Ferenčić and Karl Holubar; and *Epilogue* by Karl Holubar and Gerd Plewig (the latter again from Munich University).

The set of eye diseases presented in the book covers problems ranging from malignant melanoma to tapeworm infection, cataracts to uveitis, and hemangiomas to keratoconus. Each is richly enhanced by a clinical description and detailed differential diagnosis provided by a current authority. Stripped of their modern diagnostic armamentarium these consultants project themselves back in time when observing patients was the only diagnostic device.

Our eye is our digital camera, although a description of what we see is limited to our skill in language. For that reason this book can be seen also as some kind of experiment performed to see how original pictures of diseases from past periods will influence modern experts, how they will deal with the material visually but also intellectually following associative paths of thinking and concluding. For that reason this book is challenging the time it is not classical history neither contemporary, it is simply timeless.

The whole project was prepared within the frame-work of an inter-academy project *Iconography in Medicine*, of the Austrian and Croatian Academies of Sciences. The gallery of pictures (drawings, gouaches, prints), is a selection, taken from the files of the Vienna Institute where the images are kept since they were donated by the Department of Ophthalmology, University of Vienna, mainly in the mid-seventies of the 20th century. Hopefully we shall set eyes on some more such superb projects between the two Academies and unearth treasures stowed away in crates and boxes, destined to be forgotten.

Željko Dugac

INSTRUCTIONS TO AUTHORS

RAD Hrvatske akademije znanosti i umjetnosti – Medical Sciences is a peer-reviewed biomedical scientific journal that publishes contributions relevant to biomedicine. Manuscripts are accepted on the understanding that they are contributed to this journal alone.

General instructions for the preparation of manuscripts are given in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publications issued by International Committee of Medical Journal Editors (<http://www.icmje.org>).

Manuscripts should be sent to the Address of the Editorial Office.

RAD (ISSN 1330-5301) offers free access to full-text articles on the Portal of Scientific Journals of Croatia – HRČAK (<http://hrcak.srce.hr>). The full text of RAD can be found on EBSCO Publishing's databases.

Address of the Editorial Office
Razred za medicinske znanosti
Hebrangova 1, 10000 Zagreb, Croatia
Tel. ++ 385 1 4895 171
E-mail: vocak@hazu.hr

Rad Hrvatske akademije znanosti i umjetnosti, knj. 499

Nakladnik

HRVATSKA AKADEMIJA ZNANOSTI I UMJETNOSTI

Za nakladnika

akademik Slavko Cvetnić, glavni tajnik

Tehnički urednik

Ranko Muhek

Naklada

400 primjeraka

Tisak

Intergrafika