

REVIEWS

UDC 571.27

Allergy and Autoimmunity: Molecular Diagnostics, Therapy, and Presumable Pathogenesis

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Received December 1, 2015; in final form, April 24, 2016

Abstract—Allergic and autoimmune diseases represent immunopathological reactions of an organism to antigens. Despite that the allergy is a result of exaggerated immune response to foreign antigens (allergens) and autoimmune diseases are characterized by the pathological response to internal antigens (autoantigens), the underlying mechanisms of these diseases are probably common. Thus, both types of diseases represent variations in the hypersensitivity reaction. A large percentage of both the adult and pediatric population is in need of early diagnostics of these pathologies of the immune system. Considering the diversity of antibodies produced in allergic and autoimmune disease and the difficulties accompanying clinical diagnosing, molecular diagnostics of these pathological processes should be carried out in several stages, including screening and confirmatory studies. In this review, we summarize the available data on the molecular diagnostics and therapy of allergic and autoimmune diseases and discuss the basic similarities and differences in the mechanisms of their development.

Keywords: allergy, autoimmunity, antibodies, diagnostics, multiplex assay, immunotherapy

DOI: 10.1134/S0026893317020030

ETIOLOGY AND PATHOGENESIS OF ALLERGY AND AUTOIMMUNE DISEASES

The Role of Genotype in the Development of Disease

Researchers in the area of allergy and autoimmune diseases are extensively discussing the exact role of the genetic background in their development, whether individual predisposition to one type of disease is related to the risk of another type, and whether the presence of one immunopathology rules out the development of another [1]. Numerous studies have addressed these questions; however, no definite answer has been found so far.

It is known that some carriers of alleles that predispose an individual to a certain allergic or autoimmune disease never actually develop the condition. Other individuals are regularly exposed to environmental risk factors but do not fall ill due to the lack of the corre-

sponding genetic component. The underlying reason for this is that allergic and autoimmune diseases are typical multifactorial diseases that depend on a number of genes, as well as on different environmental influences. Only an interplay between internal and external factors triggers their development.

The role of genetic factors in these diseases was demonstrated by studying families where one or both parents had a certain immune disorder. It was found that the risk of a child developing the disease was 50% if one of the parents was diagnosed with atopy and 75% if both parents were affected [2]. Twin studies showed that the risk of a systemic autoimmune disease, e.g., systemic lupus erythematosus, was 24% for the other twin if one of the twins was affected [3]. In dizygotic twins, this risk did not exceed 2% [4]. Furthermore, it was shown that, on average, the risk of autoimmune diseases is determined by environmental factors by 30–45% [5].

A major breakthrough in the detection of genes responsible for allergy and autoimmune diseases was associated with genome-wide screening for associations between their phenotypic manifestations and single-nucleotide polymorphisms. Hundreds of studies performed for a number of diseases identified numerous genes that contribute to their development [6, 7]. The functions controlled by these genes include chemotaxis, lymphocyte activation, T-cell differentia-

Abbreviations: Treg, regulatory T cells; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; TGF- β , transforming growth factor β ; HLA, human leucocyte antigens; TNF, tumor necrosis factor; PTPN22/22, protein tyrosine phosphatase nonreceptor type 2/22; TNFAIP3, TNF- α -induced protein 3; TRAF1, TNF receptor-associated factor 1; TNFRSF5, TNF receptor superfamily member 5; Th1 and Th2, type 1 and 2 T-helper cells; IL, interleukin; IgA, IgE, IgG, IgM, immunoglobulins A, E, G, M; Fc ϵ RI, high-affinity IgE receptor; sIgE, sIgG, and sIgG4, allergen-specific immunoglobulins E, G, G4; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1, ASIT, allergen-specific immunotherapy.

tion, Treg functioning, internal immunity, adaptive immune regulation, inflammation, autophagy, barrier functions, the activation and inhibition of NF- κ B, TGF- β -dependent signaling, antimicrobial defense, phagocytosis, and others.

A central position in this list belongs to the genes of the human histocompatibility system, which encode antigen-presenting proteins located on the surfaces of T cells that enable a specific immune response [8, 9]. Presumably, as the most polymorphic region of the human genome, *HLA* genes are involved in different diseases, including autoimmune and allergic diseases [10]. For example, certain *HLA* haplotypes are associated with more than 30 different autoimmune diseases [11] and, at the same time, with allergic reactions [12]. The same *HLA* haplotype or allele can be involved in different pathological immune processes. Therefore, it can be concluded that only predisposition to a disease is inherited, while its clinical manifestations depend on other factors. On the other hand, some *HLA* alleles can act as risk factors for one type of disease and inhibit the other type. For instance, it was shown that the *HLA* allele *DRB1* plays an important role both in some allergic [12] and autoimmune [13] diseases.

According to the data from population studies, the second most important group of genes involved in autoimmune disease includes *PTPN22* and *PTPN2*, which encode tyrosine phosphatase [14] and tyrosine kinase [15], respectively, as well as *TNFAIP3*, *TRAF1*, and *TNFRSF5*, which encode proteins of the NF- κ B signaling cascade [16].

Polymorphisms of genes that encode cytokines and their receptors also contribute to the development of immunopathological reactions and diseases associated with impaired equilibrium between Th1 and Th2 lymphocytes [10]. It was shown that a polymorphism of the *IL12* gene is associated with psoriasis (a Th1-dependent disease) and atopic dermatitis (a Th2-dependent disease), while genes that encode IL-33 and its receptor are involved in the pathogenesis of bronchial asthma [17]. On the other hand, a certain gene variant (e.g., *IL-13*) can be a risk factor for one disease (for instance, allergic bronchial asthma) and at same time protect from another one (autoimmune juvenile idiopathic arthritis) [1].

In two population studies, the search for genes involved in allergies, it was found that 12 out of 18 genetic loci identified were also associated with autoimmune diseases [18, 19]. Thus, the further identification and analysis of these common loci may help elucidate the relationship between the pathogenesis of allergy and autoimmune diseases.

On the whole, the results of genome-wide association studies suggest that, on average, the contribution of genetic factors to each individual allergic or autoimmune disease is no more than 50% [5].

Role of Environmental Factors

The development of both allergic and autoimmune diseases depends not only on common genetic factors, but also on numerous external risk factors, such as infectious agents, medical drugs, or toxic compounds, which can induce the immune system to respond by hypersensitivity reactions to external antigens (allergy) or by the impaired recognition of its own internal antigens (autoimmunity) [1].

It is known that a chronic allergy is promoted by the constant presence of allergens in the environment and failure to avoid exposure. Infectious diseases and mucosal damage caused by toxic agents can increase sensitivity to certain allergens [10].

Infections can be considered to be inducers of allergies, they can both stimulate the chronic inflammatory process and play a protective role [20]. The latter effect can be explained by the fact that microorganisms can promote a switch from a Th2- to Th1-dependent immune response or stimulate the production of IL-12, an immunosuppressive cytokine.

Infections also play an important role in autoimmune diseases, but do not present major unique risk factors. Actually, no particular viral or microbial agent has been linked to any certain disease by present. Any autoimmune disease is triggered by inflammation-induced tissue damage, which can be caused by an infection, along with other factors [21]. At the same time, some evidence suggests that infections can also protect the organism from autoimmune inflammation, as it is also the case with allergies [20].

Furthermore, drugs, chemical agents, food products, UV radiation, and other agents are also risk factors that can disrupt protective barriers and facilitate their penetration by allergens, as well as cause modification of own antigens and damage the regulatory mechanisms. Sometimes, an autoimmune disease (e.g., Sjogren's syndrome) can even develop as a result of allergen-specific immunotherapy (ASIT). For instance, a case of Sjogren's syndrome was described in a patient treated with ASIT for pollinosis [22]. Before treatment, no specific autoantibodies were detected in the patients' blood, but on week 44, blood tests detected autoantibodies to extractable nuclear antigens, in particular to SS-A/Ro, and the patient developed signs of Sjogren's syndrome. Schirmer's test and the saliva test were also positive. Even though immunotherapy was discontinued immediately after detecting autoantibodies in blood, signs of Sjogren's syndrome could be observed for another year.

Mechanisms of Development of Allergy and Autoimmune Diseases

It is known that most allergic diseases are based on immediate hypersensitivity reactions. Exposure to an allergen stimulates differentiation of Th2 cells and proliferation of B cells, which produce IgE in response

to cytokines. These antibodies bind to receptors on the surface of mast cells and basophils, triggering the typical effector response (the release of histamine and heparine, prostaglandins, and leukotrienes, as well as IL-4 and IL-5) and inducing manifestations of the allergy.

Although autoimmune reactions develop via a different pathway, an increase in IgE levels was reported in systemic lupus erythematosus [23], autoimmune thyroiditis, bullous pemphigus [24], and chronic urticaria [25], as well as in different forms of vasculitis and dermatomyositis. On the other hand, some atopic allergies (e.g., atopic dermatitis) are characterized by the presence of IgE antibodies to certain human own antigens, and their level correlates with the severity of the disease [26]. Moreover, exposure to antigens, similar to own antigens (autoantigens), can cause an allergic immune response [27].

The detection of antibodies is closely related to the phenomenon of autoallergy: a pathological process that results from the production of IgE antibodies against its own antigens, which is induced by environmental allergens that bear structural or immunological similarity to human proteins. These trigger properties were described for profilin of birch pollen [28], superoxide dismutase and ribosomal P2 protein of *Aspergillus fumigatus* [29], animal albumins [30], and plant cytochromes [31]. Most commonly, IgE antibodies against these proteins are found in the blood serum of patients with atopic dermatitis [32]. Using the phage display technique, it was shown that the principal autoallergens in atopic dermatitis are Hom s 1–5 proteins, which are structurally similar to animal, plant, yeast, and bacterial proteins [33]. In this case, the concentration of autoantibodies in the blood correlated with the severity of the disease, but not the total IgE level in the patients' blood sera. Apparently, the initial event in the development of autoallergy in atopic dermatitis is tissue damage (to the skin, eyes, respiratory pathways, or gastrointestinal tract) induced by exogenous allergens, which causes the release of autoantigens and IgE production by B cells. It is possible that, due to their similarity to allergens, autoantigens stimulate the production of cross-reactive IgE antibodies. Moreover, by binding to IgE autoantibodies on the surface of mast cells, autoantigens cause the release of inflammation mediators and the immediate hypersensitivity response. At the same time, IgE-mediated autoantigen presentation by dendritic cells or monocytes can induce T cell proliferation, cytokine release, and delayed type hypersensitivity reaction [34]. The resulting antigen–antibody complexes can spread via the bloodstream and bind to all cells that possess IgE receptors, causing inflammation in different organs.

It should be mentioned separately that there are specific IgG autoantibodies against IgE and FcεRI [35]. These antibodies are even found in the blood of healthy patients, but their levels in patients with

asthma, atopic dermatitis, and some autoimmune diseases, as well as in those undergoing ASIT treatment are strongly increased. These antibodies can inhibit antigen-induced basophil activation by preventing IgE from binding to the corresponding allergen. In contrast to anti-IgE IgG antibodies used in therapy, e.g., omalizumab, these IgG autoantibodies can even interact with IgE molecules already bound to their receptors on the surface of basophils. This can partially explain the fact that the additional administration of anti-IgE antibodies did not produce the desirable therapeutic effect in patients with asthma, while patients with atopy and increased IgE levels did not exhibit clinical signs of the disease [36]. A separate important question is whether these anti-IgE autoantibodies act as allergens and induce allergic reactions by crosslinking IgE to the surface of basophils and mast cells. On the other hand, it is known that the preliminary binding to the allergen can alter the conformation of an IgE molecule and make its autoantigenic sites more exposed, which can lead to the production of pathogenic triple immune complexes causing damage to different organs and tissues [37].

Recent research has provided evidence suggesting that CD4⁺CD25⁺Foxp3⁺ Treg cells can prevent the development of both allergic and autoimmune disease [38]. For instance, Treg levels are increased in healthy individuals regularly exposed to allergens, as well as in patients with atopy after successful immunotherapy [39]. Treg cells control the immune response by suppressing the activity of immunocompetent cells, such as CD4⁺ and CD8⁺ T cells, B cells, natural killers, and antigen-presenting and mononuclear cells. Their repressor function is mediated by the release of suppressor cytokines, the inactivation of proinflammatory IL-2, cytotoxicity, and the inhibition of costimulation and antigen presentation. Furthermore, Treg cells regulate the production of antibodies against their own and foreign antigens by arresting Th cell differentiation [40, 41].

An important role in the development of allergic and autoimmune diseases belongs to highly conserved TIM proteins (for T-cell immunoglobulin and mucin domain) expressed on many types of immune system cells. In humans, the *TIM* gene family comprises three members, i.e., *TIM-1*, *-3*, and *-4*. It is known that the interaction of TIM-1 with TIM-4, as well as with TIM-3, induces an immune reaction along the Th2 pathway and, thus, promotes asthma and allergic rhinitis. It was also shown that large quantities of TIM-3 ligands are present on gut epithelial cells in patients with food allergy, whereas TIM-4 can prevent allergic reactions. In addition, TIM-1 overexpression was observed in mononuclear cells of patients with systemic lupus erythematosus, and TIM-3 was shown to play a protective role in multiple sclerosis and rheumatoid arthritis [42].

The second type of pathological immune reactions (type-II hypersensitivity) involved in the pathogenesis of both allergies and autoimmune diseases is mediated by IgG and IgM interaction with cell surface antigens. Their potential targets are erythrocyte-bound antibiotics (penicillin), rhesus-D antigen, or components of the basal membrane. These antigen-antibody complexes can activate components of the complement system, which results in cell opsonization, lysis, or phagocytosis. This mechanism underlies, e.g., the development of drug allergies, as well as a number of autoimmune diseases (autoimmune cytopenia, Goodpasture syndrome, Basedow's disease, myasthenia gravis). Moreover, cytotoxic IgG antibodies can specifically contribute to the pathogenesis of some atopic conditions, such as bronchial asthma [43].

Type-III hypersensitivity reactions result from the production of immune complexes due to the interaction of either external or internal antigens with IgG or IgA antibodies. These complexes are not eliminated from the organism, but accumulate in body tissues (blood vessel walls, renal glomeruli, synovial fluid), causing inflammation and organ damage. This is the mechanism that underlies the development of an overwhelming majority of autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis, and others), as well as of some allergies (serum disease, alveolitis) [1].

Type-IV immunopathological reaction (delayed hypersensitivity) is mediated by cytotoxic Th1 lymphocytes and subsequent cytokine activation, which triggers the inflammatory response and production of cell infiltrates. Among the conditions that develop along this pathway, there are both allergies (contact dermatitis) and autoimmune diseases (multiple sclerosis, type-1 diabetes mellitus) [1]. Thus, certain mechanisms of autoimmune reactions seem to correlate with the pathogenesis of allergic diseases, whereas chronic allergy can acquire some traits of an autoimmune process [44].

LABORATORY DIAGNOSTICS OF ALLERGIC DISEASES

The golden standard of allergy diagnostics that makes it possible to detect hypersensitivity to a particular allergenic agent is provocation trials, i.e., nasal, conjunctival, inhalation, and sublingual tests. However, taking into account numerous contraindications and the risk of systemic reactions, provocation trials are only performed when patient's history data disagree with the results of safer *in vivo* skin tests (these are not performed in infants, subjects with skin diseases that interfere with evaluating test results, or individuals prone to developing systemic reactions).

When skin tests are impossible, concentrations of sIgE to putative allergenic agents can be determined using *in vitro* assays. It should be pointed out that

increased levels of allergen-specific IgE only confirm type-I hypersensitivity in the presence of the corresponding clinical signs.

sIgE levels can be determined using direct and reverse immunoassays. Direct immunoassay is based on the binding of sIgE antibodies from a blood serum specimen to an allergen adsorbed or covalently linked to the solid phase (support). The resulting allergen-sIgE complexes are detected using species-specific anti-IgE antibodies conjugated to a fluorescent label or an enzyme for subsequent chemoluminescent analysis. Variants of direct immunoassay can be performed using the ImmunoCAP FEIA system (Phadia), where allergens are placed in special cap wells that contain material with well-developed surfaces, as well as on plates with cellulose disks that carry adsorbed antigens. These methods enable the quantitative evaluation of sIgE levels and are usually compatible with fully automated analytic systems. They exhibit high sensitivity and specificity, but are poorly appropriate when sensitivity screening for a large number of allergens is necessary, since the volume of serum required for the analysis, as well as its cost, grow proportionally to the number of allergens tested.

Multiplex methods based on the immunoblotting technique do not have this shortcoming. Blood serum specimens are analyzed using cassettes with nitrocellulose membrane strips carrying allergens. During the test, serum sIgE bound to the antigen on the membrane form complexes with enzyme-anti-IgE conjugates in the detecting antibody solution. Upon substrate addition, a stained product appears, and the staining intensity depends on the sIgE concentration. The first test systems based on immunoblotting were proposed for semiquantitative express analysis of sIgE antibodies to small (no more than ten) allergen panels. Currently, with the advent of more precise and sensitive scanning devices, it has become possible to perform quantitative sIgE analysis for considerably larger numbers of allergenic agents [45].

The biochip technology offers nearly unlimited possibilities for enlarging the allergen panel for the constant minimal volume of serum specimens. Biochips are matrices of cells that contain different immobilized probes. Similar to the oligonucleotide chip technique, protein probes are immobilized on a solid phase, i.e., a modified glass or plastic surface, or in gel structures [46].

One of the very first developments in the area was a 2D biochip that could be used to determine the concentrations of sIgE to four allergen extracts [47]. However, because of their low analytic sensitivity (1 IU/mL compared to 0.1 IU/mL for ImmunoCAP), although these biochips are convenient, they are less efficient than classic microplate-based immunoassay techniques.

Biochips that employ different solid phases and methods of signal amplification were described in [48–51]. Using the hydrogel biochip technology

(Engelhardt Institute), an allergochip was developed for quantitative analysis of a 21-allergen preparation (extracts and recombinant proteins) [52]. The Micro-test Dx platform designed using the biochip technology [53] enables simultaneous analysis of sIgE to both recombinant proteins and extracts. Antibodies to 22 allergen extracts and four recombinant components are determined in a semiquantitative assay.

The only commercially available biochip for molecular diagnostics of allergy, ImmunoCAP ISAC (Phadia) enables semiquantitative analysis of sIgE to 139 individual natural and recombinant allergens. The ImmunoCAP ISAC panel was expanded for the purposes of MeDALL, a European research program for allergic diseases in children. The proposed MeDALL-chip with modified panels of inhaled and food-borne allergens exists in two variants: one of them contains a considerably broader range of bacterial superantigens, and the other one was supplemented with additional food-borne allergens for studying hypersensitivity to wheat [54].

The principal disadvantage of biochips, as well as of some other direct multiplex techniques, is that sIgE and sIgG compete for binding to allergen, which may lead to false negative results for one of these antibodies. Reverse immunoassay methods are free of this shortcoming, since species-specific anti-IgE antibodies immobilized on the support only form complexes with sIgE present in serum specimens. sIgE binding to an allergenic agent is evaluated using liquid biotinylated allergens. Modern methods of determining sIgE are fairly sensitive, and the obtained results correlate well with skin tests; therefore, they are widely used in the diagnostics of type I hypersensitivity [55, 56].

The effector cells that participate in different types of allergic reactions are basophils and mast cells. For this reason, *in vitro* techniques have been developed to detect proinflammatory mediators released as a result of their degranulation in the presence of a sensitizing allergen. In particular, their number includes a test for the specific release of histamine as a primary mediator of inflammation [57], as well as the cellular allergen stimulation test (CAST), which determines leukotrienes and secondary inflammatory mediators produced by oxidation of arachidonic acid [58]. In the cell-based basophil activation test (BAT), the expression of CD63 and CD203c activation markers on the surface of basophils following antigen-specific stimulation *in vitro* is registered by flow cytometry [59].

In vivo and *in vitro* diagnostics of allergic reactions is performed with material obtained from the allergen source, usually with allergen extracts. Each extract is a mixture of proteins, glycoproteins, polysaccharides, and contains both unique components characteristic of this particular extract and components homologous to proteins of other extracts, which are responsible for cross reactions. If the sensitivity to a preparation is due to the presence of sIgE to its major components char-

acteristic for the particular allergen (or a group of allergens), it is described as the true sensitization to the allergen. On the other hand, if sensitivity is associated only with a reaction to cross-reactive components of the allergen, it is referred to as cross-sensitization.

The concept of molecular (component-resolving) diagnostics was developed to enable identification of the primary source of allergy and the optimal choice of the ASIT agent based on obtaining sensitization profiles for known individual allergens, recombinant proteins, or highly purified extract components. This component-resolving diagnostics can be successfully performed using the ImmunoCAP ISAC system by Phadia described above [54].

LABORATORY DIAGNOSTICS OF AUTOIMMUNE DISEASES

In an autoimmune disease, the patients' blood serum contains several types of autoantibodies, the diagnostic significance of which is significantly increased if they can be assessed simultaneously [60–62]. For instance, more than 100 different autoantibodies have been described in patients with systemic lupus erythematosus [63].

Serological tests for detecting circulating autoantibodies play an important role in the laboratory diagnostics of autoimmune diseases. They are used to evaluate the intensity of the process and the disease prognosis or to diagnose the disease at an early stage and in individuals who are still asymptomatic [64]. However, the detection of autoantibodies in the absence of clinical signs does not suffice for diagnosing autoimmune disease [65]. For the differential diagnosis, it is necessary to determine antibodies with different specificity and lymphocytes sensitized against antigens of affected tissues, as well as to detect immune complexes [66].

The most important tests include the evaluation of antibodies to nuclear antigens, Sm-antigen, dsDNA, and cardiolipins for the diagnosis of systemic lupus erythematosus [67, 68], of autoantibodies to β 2-glycoprotein I and to cardiolipin in antiphospholipid syndrome [69]; of rheumatoid factor IgM/IgA, antibodies to citrullinated proteins and to mutated citrullinated vimentin in rheumatoid arthritis, and of anti-Ro and anti-La antibodies in Sjogren's syndrome [70]. In some cases, autoantibodies have prognostic significance because their concentrations change at different stages and with different course of the diseases, as was shown for anti-dsDNA autoantibodies in systemic lupus erythematosus [71] and for antineutrophilic cytoplasmic autoantibodies (ANCA) in ANCA-associated autoimmune vasculitis [72]. It has also been demonstrated that autoantibodies can be detected several years before the clinical manifestation of the disease and, therefore, can be used in surveys analyzing the groups of risk for autoimmune diseases [73].

The golden standard used to determine the antinuclear factor [74], antacentromeric antibodies, antibodies to extractable nuclear antigens, and some nonspecific antibodies is the indirect immunofluorescence technique, which involves identifying cell compartments that exhibit major fluorescence after binding with autoantibodies from the patient's blood serum. This method is highly sensitive and can be used to determine more than 100 autoantibody targets. However, it also has certain limitations due to artifacts of cell fixation. To avoid false negative results, this method is used in combination with ELISA, which is employed to determine antibodies to dsDNA, nuclear and cytoplasmic antigens, citrullinated peptide, and other antigens [75, 76].

In contrast to monoplex test systems, multiplex techniques enable the simultaneous detection of autoantibodies to several targets, i.e., individual autoantibody profiling. In addition, these test systems are more time- and cost-efficient than monoplex systems, and can be performed with smaller sample volumes. They have been developed both in the planar form (immunoassays performed on microchips and nitrocellulose membranes) and for 3D immunoassays (on microbeads and nanoparticles).

Planar microchips are spots of DNA or protein molecules loaded on slides, polystyrene microplates, or nitrocellulose membrane. The first microchip platform for autoantibody analysis was described by Joss et al. [77]. It could be used to simultaneously determine 18 known autoantibodies using immobilized antigens and subsequent chemoluminescence detection of the resulting complexes. Currently, commercial test systems based on planar microchips have been proposed by SQI Diagnostics, Canada (Ig-Plex), and ImmunoConcepts, United States (Microzyme). There are also microchips for analyzing secreted proteins and inflammation markers, such as cytokines, chemokines, metalloproteinases, and growth factors. Their detection often plays an auxiliary role and serves to evaluate the efficacy of treatment, in particular of rheumatoid arthritis and relapsing polychondritis [78, 79] with targeted monoclonal antibodies [80].

In addition to microchips with immobilized protein antigens, the diagnostics of autoimmune diseases can employ DNA microchip variants that carry different oligonucleotides as probes. Swapan et al. used this DNA microchip to identify 13 genomic loci that contribute to the susceptibility to systemic lupus erythematosus [81]. A study by Bennett et al. determined a combination of interferon-encoding genes exhibiting a high level of correlation with the same disease [82]. However, this approach cannot so far be fully recognized because of the lack of standardization in microchip production, the limited choice of genes to be tested, and the use of suboptimal methods of statistical analysis.

Another popular tool in the laboratory diagnostics of systemic diseases are test systems employing linear immunoblotting on nitrocellulose membranes with a colorimetric detection system [83, 84]. They are most commonly used to determine serum autoantibodies to nuclear and cytoplasmic antigens and can detect up to 20 different analytes in a single test. Important advantages of this method are its rapidity and high specificity.

Most nonplanar multiplex test systems employed for the diagnostics of autoimmune diseases utilize the xMAP® technology (Luminex, United States), including flow cytometry using fluorophore-labeled antigen-carrying polystyrene microbeads [85]. The use of specific ratios of fluorophore concentrations gives rise to more than 100 types of particles, each with its own unique spectral profile. To detect a certain analyte, microbeads are first incubated with the specimen, and next with the streptavidin–phycoerythrin conjugate, which then binds to the biotin label on the detection antibodies. The fluorescence intensity of the resulting complex is proportional to the concentration of the analyte in question. The UltraPlex® technology (SmartBead Technologies Ltd., Great Britain), which employs antigen-carrying bar-coded nanoparticles for profiling antinuclear antibodies, is also worth a special mention.

An approach based on chromatography and mass spectrometry, in particular surface-enhanced laser desorption-ionization with time of flight (SELDI–TOF) analysis, can be used to selectively determine autoantibodies following minimal sample-preparation procedure. This method requires complex equipment, but enables the detection of the whole autoantibody repertoire, even in small specimen volumes [86]. This method is especially efficient for proteins with a molecular weight of less than 30 kDa. For instance, the MALDI–TOF/TOF platform was used to analyze peptides, the altered serum levels of which identifies them as novel biomarkers, in particular biomarkers of autoimmune diseases [87].

Despite the considerable progress in the diagnostics of autoimmune diseases, test systems that would enable simultaneous analysis of 30–50 autoantibodies with the highest clinical significance have still not been developed.

THERAPY OF ALLERGY AND AUTOIMMUNE DISEASES

The principal approach to preventing allergic disease implies that the patient should avoid contacts with the allergen. Symptomatic treatment employs medications that neutralize the biologically active compounds release, block H1 histamine receptors on the surface of target cells, or increase the resistance of membranes to damaging agents, including nonallergenic agents. The only available pathognomonic treatment is ASIT.

Traditional immunotherapy involves regular intracutaneous or sublingual administrations of a preparation that contains the inducer allergen [88]. At the early stage of treatment, this leads to the suppression of effector cells as a result of the repeated partial release of inflammatory mediators below the threshold of basophil and mast cell activation associated with a systemic anaphylaxis reaction [89].

The subsequent development of immunological tolerance involves the differentiation of CD4⁺CD25⁺ T cells into allergen-specific CD4⁺CD25⁺Fox3⁺ Treg cells and a reduction in the population of allergen-specific Th2 cells [90], which apparently results in a decrease in sIgE levels accompanied with an increase in the levels of sIgG, in particular of sIgG4 [91], which acts as blocking antibodies.

The most commonly used ASIT preparations are based on allergen extracts. However, since the composition of these extracts can vary considerably, the therapy may sometimes produce unpredictable results. The advent of vaccine preparations based on individual purified or recombinant proteins may provide a solution to this problem.

To reduce the intensity of the immediate immune response after ASIT administration, vaccines have been developed based on recombinant allergens that induce lower IgE reactivity due to partially modified epitope specificity of allergen molecules. These modifications involve the elimination of conformational epitopes by destroying disulfide bonds [92], allergen fragmentation [93], or oligomerization [94], or else by introducing point mutations into known functional domains. ASIT, which employs short synthetic peptides that contain immunodominant epitopes responsible for interactions with antigen-specific Treg cells, is also considered to be an efficacious option [95].

Vaccines designed using the haptene-carrier principle have also proved efficient. Following an epitope analysis for different recombinant antigens, peptides of 25–40 amino acid residues are synthesized so as to reproduce the sequence of the conformational epitope of the molecule. Next, the peptide is conjugated to a carrier protein, usually of viral origin. Since the peptide has the same sequence as the site of allergen binding to sIgE, the administration of these vaccines induces the production both of IgG antibodies to the carrier protein and of blocking allergen-specific IgG [96].

Although ASIT alters the patient's immune response to the antigen and, thus, alleviates the clinical manifestations of the disease, vaccines are unable to completely restore the immune status, which is characteristic of healthy individuals.

In autoimmune diseases, the immune system reacts pathologically to own antigens, which are obviously impossible to eliminate from the organism. Therefore, the variety of methods proposed for their treatment is limited, the preferable options being immunosuppressive drugs (e.g., corticosteroids and

cyclosporin A) or therapeutic monoclonal antibodies. The clinical practice is making increasing use of antibodies against cytokines, including anti-TNF antibodies (Infliximab®) [97] for treating rheumatoid arthritis, Crohn's disease, and ankylosing spondylitis, and anti-IL-6 antibodies (Tocilizumab®) [98] for rheumatoid arthritis and neuromyelitis optica. Despite the large number of side effects related to a decrease in the general immune status and the resulting increased risk of infections, this approach nevertheless seems rather efficient. Interferon alpha blockers are currently undergoing clinical trials, and preparations that contain anti-CD20 antibodies and blockers of CTLA-4 and PD-1 as immune checkpoints are considered as promising therapeutic agents [99].

It is currently discussed whether ASIT, which has proved efficacious in allergic diseases, could also be employed in order to restore the immunological tolerance in autoimmune diseases. It was shown that increasing doses of antigen introduced subcutaneously increase the level of immunological tolerance and inactivate IL-10-secreting CD4⁺ Treg cells [100]. These results justify the use of increasing antigen doses and helped identify the immunological profiles and combinations of transcription factors that can serve as markers of successful immunotherapy. The introduction of these methods in clinical practice may help eliminate the need to use immunosuppressive drugs with numerous side effects in patients with autoimmune diseases.

ACKNOWLEDGMENTS

The authors are grateful to anonymous reviewers for valuable conceptual critical comments.

For the financial support of this work, the authors are grateful to the Russian Science Foundation (grant no. 14-50-00060), OOO Kompaniya Helikon, OOO AnalitLab, OOO RUSKHIMBIO, OOO BIOCHIP IMB, and the Federal Agency for Scientific Organizations.

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Translated by D. Timchenko