Avtoimunske bolezni: diagnostični izziv

Autoimmune diseases: a diagnostic challenge

Avtor / Author

Ustanova / Institute

Artur Pahor[1,2]

[1]Univerzitetni klinični center Maribor, Klinika za interno medicino, Oddelek za revmatologijo, Maribor, Slovenija, [2]Univerza v Mariboru, Medicinska fakulteta, Katedra za interno medicino, Maribor, Slovenija

[1]University Medical Centre Maribor, Division of Internal Medicine, Department of Rheumatology, Maribor Slovenia, [2]University of Maribor, Faculty of Medicine, Department of internal medicine, Maribor, Slovenia

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Prim. doc. dr. Artur Pahor, dr. med.
Univerzitetni klinični center Maribor,
Klinika za interno medicino, Oddelek za revmatologijo, Ljubljanska 5,
SI–2000 Maribor, Slovenija
Telefon +386 23212400
Faks +386 23322128
E-pošta: artur.pahor@ukc-mb.si

Izvleček

Imunski sistem nas varuje pred številnimi dejavniki okolja, ki lahko poškodujejo človeška tkiva. Deluje usklajeno in je sposoben razlikovati med človeku lastnimi in tujimi strukturami. Kadar prepoznava lastna tkiva kot tuja, se sprožijo vnetni mehanizmi proti lastnim tkivnim in celičnim strukturam. Te strukture imenujemo avtoantigeni, nanje se tvorijo protitelesa. Kadar prizadene avtoimunsko vnetje pomembne organske sisteme, govorimo o avtoimunskih boleznih. Te so lahko omejene, in sicer le na en organski sistem, ali sistemske, kjer je prizadetih več organskih sistemov.

V prispevku obravnavamo najpomembnejše mehanizme, ki privedejo do avtoimunskega vnetja, v nadaljevanju pa tudi diagnostiko avtoimunskih bolezni, ki temelji predvsem na klinični sliki. V veliko pomoč pri diagnozi je tudi določevanje nekaterih specifičnih

Abstract

The immune system defends humans against foreign factors that could damage healthy tissue. It detects and distinguishes a wide variety of substances from the organism's own structures. Failing to recognize the organism's own structures as self-structures causes autoimmune disorders. These self-structures are called "autoantigens". Autoimmune disorders are divided into those directly damaging only a single organ system, and those in which many important organs are damaged (systemic). This article deals with the most important autoimmune mechanisms inducing autoimmune inflammation. It discusses the diagnoses of autoimmune disorders, which are based mainly on the clinical picture, and the most frequently used laboratory and immunological tests crucial for diagnosing particular autoimmune diseases.

protiteles na avtoantigene. Navedene so najpogostejše laboratorijske preiskave in tisti imunološki testi, ki so pomembni za diagnozo posameznih avtoimunskih bolezni ter sodijo tudi med diagnostične kriterije.

Poudarjeno je, da je zlasti v družinski medicini potrebno biti pozoren na klinične znake avtoimunskega vnetja, poznati pa je potrebno tudi imunološke laboratorijske teste, saj je pravočasna diagnostika bistvena za zdravljenje avtoimunskih boleznih.

The purpose of this article is to emphasize the importance of the early clinical picture and laboratory testing of autoimmune diseases.

INTRODUCTION

The immune system protects humans against numerous factors from the environment which could potentially damage cells and tissues. The main characteristic of this system is the ability to differentiate native cell and tissue structures from foreign organisms. T- and B-lymphocyte receptors have a decisive role in this process. Errors in identification lead to failure in recognizing native structures and consequently inflammatory reactions to native tissues, leading to autoimmune disease. The main characteristic of autoimmune diseases is an immunological inflammatory reaction of the organism against native tissues, consequently damaging them. The term "autoimmune" indicates the presence of antigens and T-lymphocytes (which identify native structures as antigens). They are referred to as "autoantigens". This process is not always destructive. It is often activated in different infectious diseases in a limited condition, and is also self-limited. If this not occurs, native tissues are damaged and the disease process is initiated.

MECHANISMS OF AUTOIMMUNE PROCESSES

The foundations of these mechanisms were laid out in 1900 by Paul Ehrlich who proved the existence of factors inhibiting inflammatory reactions against native tissue. Burnet proved his theory of clonal selection by showing that interactions between lymphoid cells and their specific antigen during foetal development lead to the elimination of "forbidden clones" (self-reactive lymphocytes), which could lead to autoimmune reactions during development. Several authors proved that immunization alone can trigger autoimmune disease and therefore the existence of autoantigen-binding cells in the circulation. A limited autoimmune response in numerous infectious diseases has also been confirmed by several authors. All of these observations led to the discovery that cell clones reacting to antigens are also present in healthy people. Processes of clonal deletion which inhibit the activation of these cells have also been demonstrated (1). Maintaining non-reactivity to autoantigens is possible with three processes: (i) sequestration of native antigens, thereby isolating them from the immune system; (ii) specific non-reactivity (tolerance or anergy) of relevant T cells and B cells; and (iii) limiting the chance of potential reactions with autoantigens using regulatory mechanisms. Disturbance in these processes leads to autoimmunity, which is primarily linked to stimulation with exogenic factors (viral or bacterial antigens) or with endogenic errors in the cells of the immune system. If these factors lead to expression of the receptors of autoantigen-reactive T and B cells, the autoimmunity processes are initiated.

Known exogenic factors are molecular mimicry as well as cross-reactions between microbial products and native antigens, leading to the activation of auto-reactive lymphocytes (2). The most familiar case of autoreactivity caused by molecular mimicry is rheumatoid fever, in which the streptococcal M protein cross-reacts with myosin, laminin, and other matrix proteins. Molecu-

lar mimicry between microbial proteins and native tissues has been identified in systemic sclerosis, rheumatoid arthritis, and type-1 diabetes mellitus. It has also been established that certain bacterial products (e.g. endotoxins) function as adjuvants to autoantigens, which are transformed into autoimmunogens (1).

Endogenic factors affecting the development of autoimmunity are primarily: changes in antigen presentation; surplus of antigen-specific T-helper cells; increased stimulation of B-cells and their consequent surplus; disturbances in programed cell death (apoptosis); lack of balance between pro-inflammatory and inhibitory inflammatory cytokines; and changes in immunoregulatory mechanisms (mostly related to disturbances in the activity of T-cell expression). The latest data related to the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome also suggests this. In this syndrome, an error occurs in expression of the gene responsible for the molecular synthesis required for the differentiation of regulatory T-cells. It was also demonstrated in animal models that injecting normal regulatory T-cells prevented the development of autoimmunity in animals with the IPEX syndrome (3).

Several other factors affect the incidence of autoimmunity. Gene predisposition, linked to the major histocompatibility complex (MHC) is certainly one of them, along with age (certain autoimmune phenomena are more frequent in younger people and in older age), sex, and sexual hormones (certain autoimmune diseases are more common in younger women). Numerous microbial- and physical-chemical factors from the environment (e.g. ultraviolet light) can trigger autoimmune inflammatory reactions or prevent their self-limitation (1).

IMMUNOPATHOGENESIS OF AUTOIMMUNE DISEASES

Autoimmune processes leading to tissue damage in autoimmune diseases can be divided into two major groups: (i) processes associated with autoantigens and (ii) processes associated with T-cells.

Numerous processes are associated with autoantigens, and they affect different target molecules or receptors. The blocking process or inactivation of the nicotine-acetylcholine receptor is characteristic of mystenia gravis. The same process blocking phospholipid-beta 2-glycoprotein 1 complex is present in anti-phospholipid syndrome. Blocking the insulin receptor is characteristic of insulin-resistant diabetes. The process of antibody stimulation targeting proteinase-3 is characteristic of Wegener's granulomatosis (4). In Graves' disease, stimulation is targeted at the thyroid-stimulating hormone (TSH) receptor and, in pemphigus, towards epidermal cadherin (1). Complement activation targeting the alpha chain of type -IV collagen is characteristic of Goodpasture's syndrome (1). The formation of immune complexes targeting double-stranded DNA is characteristic of systemic lupus erythematosus (SLE) (5) but, if targeting immunoglobulin (Ig) molecules, it is characteristic of rheumatoid arthritis (6). Opsonization against thrombocytes is characteristic of autoimmune thrombocytopenic purpura, but opsonization against Rh antigens is characteristic of autoimmune haemolytic anaemia (7). Antibody-dependent cell toxicity against thyroglobulin is characteristic of Hashimoto's thyroiditis (8). Several antibodies have major diagnostic value but they cannot be associated directly with the onset of autoimmune diseases. T-cell processes are associated with the production of various cytokines. Such processes have been observed in rheumatoid arthritis, multiple sclerosis, and type-1 diabetes (1).

AUTOIMMUNE DISEASES

In numerous diseases, certain elements of autoimmunity can be established, which does not necessarily indicate an autoimmune disease. To confirm with certainty that an autoimmune disease is present, one must prove that an immune response targeted at host antigens is responsible for the observed pathological processes. Detection of antibodies targeting host tissues does not suffice for the diagnosis because such antibodies are also identified in certain types of injuries and numerous infectious diseases. Therefore, one must prove that autoimmune processes are causing the observed pathological changes.

Several researchers have established that in cases in which antibodies are pathogenic, an autoimmune disease can be diagnosed if the antibodies are transferred in an animal model. Graves' disease is one such ex-

ample. Authors have also discovered that numerous antibodies can be transferred from mother to child during foetal development and cause a disease in the child which usually disappears when the amount of the mother's antibodies is decreased in the child. An exception is the congenital heart block caused by the mother's Ro antibodies (typical for SLE and Sjögren's syndrome), which does not disappear (1). Based on the information mentioned above, the criteria for determining an autoimmune disease have been defined and are shown in Table 1.

Table 1: *Autoimmune diseases: criteria for confirming the pathogenesis of immunological disease*

Major criteria
1. Presence of autoantibodies or cell activity to self-antigens
2. Proof of autoantibodies or lymphocyte infiltration in pathological tissue
3. Demonstration of autoantibodies or T-cells causing tissue lesions (transplacental transmission, transmission to laboratory animals, and in- vitro influence on cell function)
Additional criteria
1. Evidence arising from tests carried out on animals
2. Beneficial effect of immunosuppressive treatment
3. Other laboratory-based evidence of autoimmune inflammation
4. Absence of infectious or any other chronic disease

Autoimmune diseases are limited to a single organ or are systemic. Limited forms of autoimmune diseases have, in general, fewer antibodies targeting one body system. Graves' disease is a representative example in which thyroid antibodies can be established in most cases. Patients with limited forms of autoimmune diseases often tend to produce numerous antibodies which are, in general, not pathogenic to them. Patients with myasthenia gravis often have antinuclear antibodies, rheumatoid factor, thyroid antibodies and polyclonal hypergammaglobulinemia. This is usually attributed to genetic similarities in patients with autoimmune diseases. It is a known that patients with a limited form of autoimmune disease are often more susceptible to developing one more limited autoimmune disease than healthy people. One familiar case

Table 2: *Common autoimmune diseases*

Limited types of autoimmune diseases
Graves' disease
Hashimoto's thyroiditis
Type-1 diabetes mellitus
Insulin-resistant diabetes mellitus
Immune-mediated sterility
Immune-mediated Addison's disease
Pemphigus
Dermatitis herpetiformis
Autoimmune alopecia
Vitiligo
Autoimmune haemolytic anaemia
Autoimmune thrombocytopenic purpura
Pernicious anaemia
Myasthenia gravis
Multiple sclerosis
Guillain–Barré syndrome
Rheumatic fever
Sympathetic ophthalmia
Goodpasture's syndrome
Systemic autoimmune diseases
Systemic lupus erythematosus
Rheumatoid arthritis
Systemic necrotizing vasculitis
Wegener's granulomatosis
Anti-phospholipid syndrome
Sjögren's syndrome

is patients with autoimmune thyroid disease who also often develop pernicious anaemia (1).

Systemic autoimmune diseases differ from limited forms of autoimmune diseases with regard to pathological lesions, which are to be found in different organs and tissues. It is a characteristic of these diseases that autoimmune phenomena are aetiologically related to pathological changes in different tissues. An example of such a disease is SLE, in which the largest

number of autoimmune phenomena can be observed. SLE can affect almost all body systems. It is related to the presence of numerous antibodies. Their production results from generalized hyperactivity of the humoral arm of the immune system. In addition, SLE has a characteristically increased B-cell reaction, polyclonal hypergammaglobulinemia, and increased antibodies titres to certain micro-organisms (especially viruses) (5). Some of the most common autoimmune diseases are presented in Table 2.

DIAGNOSES OF AUTOIMMUNE DISEASES

The diagnosed of autoimmune are based mainly on the clinical picture, which can be very diverse (especially in systemic autoimmune diseases). Therefore, precise diagnostic criteria have been created involving clinical features and certain immunological laboratory tests. The combination of those parameters increases the diagnostic probability because the clinical signs in systemic autoimmune diseases appear to be non-specific and also present in numerous other chronic diseases (e.g. infectious, oncological). Moreover, it is well known that the findings of immunological laboratory tests can be positive in certain healthy individuals, patients with chronic infectious diseases (e.g. hepatitis, tuberculosis), and patients with myeloproliferative disorders. Therefore, the presence of antinuclear antibodies or rheumatoid factors alone does not indicate autoimmune disease (9).

Autoantbodies are targeted against several antigens. They can be cell-specific (e.g. against erythrocytes and thrombocytes) or non-cell-specific (targeted against cell components such as nucleic acids, proteins, compound lipids). There is no "ideal" screening test for the detection of autoantibodies (10). The most effective screening tests used also in our hospital are: (i) immunofluorescence test for autoantibodies against antigens of cell components - HEP-2 test, also called the antinuclear antibodies (ANA) test; and (ii) test for autoantibodies targeting antigens of tissue extracts (anti-ENA test) undertaken by enzyme-linked immunoassay (ELISA) and by radial immunodiffusion.

Special liver cells are used to carry out the HEP-2 test, in which autoantibodies react with antigens on HEP-2 cells. The ANA test is a part of the HEP-2 test because only antinuclear antibodies are being observed. It is an immunofluorescence test using a special fluorescence microscope for observing various types of immunofluorescence. To date, >25 immunofluorescence patterns have been detected. In the event of a positive ANA test our research team defines the cut-off titre of 1:160 under the fluorescence microscope as well as the type of immunofluorescence. Upon specific request an exact titre can be defined. The most common immunofluorescent samples and possible relationships to disease entities are presented in Table 3 (10, 11).

Table 3: *Major immunofluorescent HEP-2 test samples*

Location: **nucleus (antinuclear antibodies – ANA)**

Fluorescence pattern	Possible antigens	Possible diagnosis
Homogeneous	DNA	SLE
Speckled	NOR	PBC
Nucleolar	RNA polymerase, fibrillarin	SSc, myositis
Centromere	Centromere proteins	Limited SSc

Location: **dividing cel**

Fluorescence pattern	Possible antigens	Possible diagnosis
Centriolar	Centriolar proteins	SJS
Centriolar proteins	Spindle fibres	SJS, malignoma

Location: **cytoplasm**

Fluorescence pattern	Possible antigens	Possible diagnosis
Equally dense	RNA synthetase, ribosomes	Myositis, SLE
Smooth-muscle-like	Actin	Autoimmune hepatitis
Mitochondrial	PDH	PBC
Golgi apparatus	GA proteins	SLE, RA, SSc

SLE = systemic lupus erythematosus, NOR = nucleolus organizer region, PBC = primary biliary cirrhosis, SSc = systemic sclerosis, SjS = Sjögren's syndrome, PDH = pyruvate dehydrogenase complex, GA = golgi apparatus, RA = rheumatoid arthritis

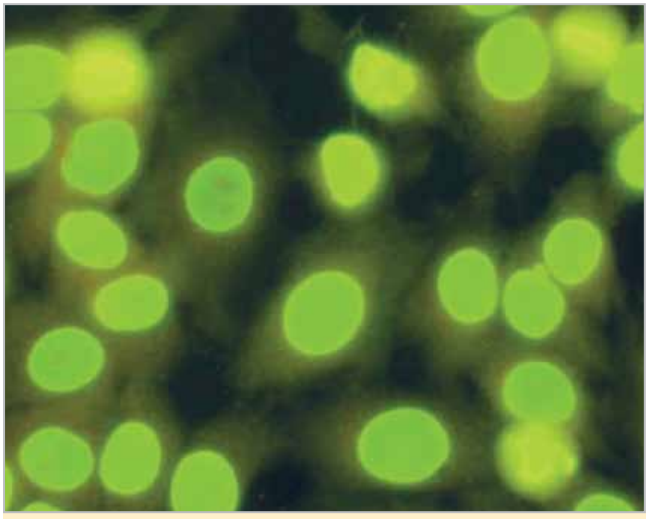

Figure 1. *ANA test: homogeneous pattern of immunofluorescence (with permission: Dept. for laboratory diagnostics UKC Maribor)*

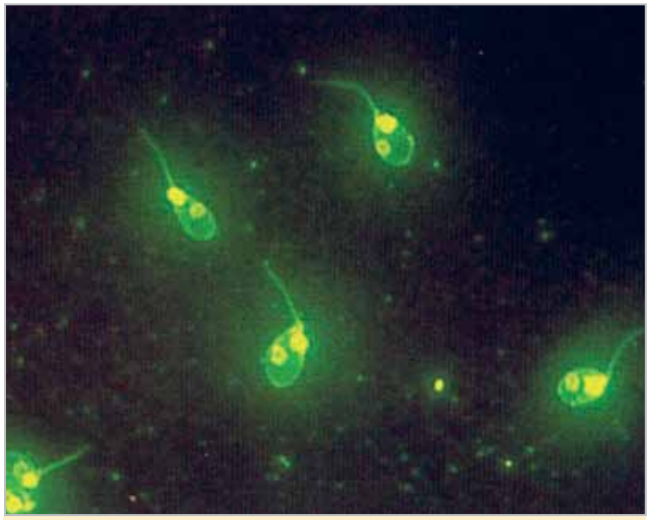

Figure 2. *Staining for antibodies against double-stranded DNA (on protozoa Critidia luciliae) (with permission: Dept. for laboratory diagnostics UKC Maribor)*

It is a highly sensitive test but with very low specificity. A positive ANA test at low titre can also be found in healthy individuals, as well as in numerous chronic conditions. The prevalence of a positive ANA test in healthy individuals is ≤30%, more common in females, and increases with age. It is only a diagnostic test and is not related to active disease. In the event of a positive ANA test, our research team also undertakes the anti-ENA test, as recommended by the well-known rheumatologist Dr Martin Fritzler (12). ELISA is the method of choice for defining specific autoantibodies, which is part of the diagnostic criteria for individual autoimmune diseases. The following antibodies are defined by the ELISA method: Ro or SS-A, La or SS-B, PCNA, Sm, antihistone antibodies, antiribonucleoprotein (RNP), Jo-1, and scl-70. The most common diseases associated with the autoantibodies mentioned above are shown in Table 4 (10).

Table 4: *Major autoantigens and disease entities (%)*

Antigen	Autoimmune disease
Sm	SLE (»30%), rarely MCTD
RNP	MCTD (≤95%), rarely SLE
Ro	SLE (20–60%), SjS (40–90%)
La	Rarely SLE and SjS (40–90%)
PCNA	Rarely SLE
Jo-1	PM, dermatomyositis (30%)
Scl-70	SSc (≤70%)
Ku	PM, rarely SLE, SSc

SLE = systemic lupus erythematosus, SSc = systemic sclerosis, SjS = Sjögren's syndrome, PM = polymyositis, PCNA = proliferating cell nuclear antigen, MCTD = mixed connective tissue disease, RNP = ribonucleoprotein

Antibodies against double-stranded DNA are highly specific for SLE, and observed in »60% of SLE patients. The amount of antibodies is dependent upon disease activity (13).

Anti-phospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, and antibodies against beta-2-glycoprotein I) are crucial for defining the anti-phospholipid syndrome, are associated with coagulation disorders, thromboembolic events, and pregnancy complications (abortion, stillbirth) (14).

Rheumatoid factors (RFs) are antibodies against the Fc fragment of IgG. They can be subdivided as IgG, IgM or IgA, and are used as diagnostic markers for rheumatoid arthritis (RA). A higher titre might predict a severe disease course. Especially low titres might indicate other autoimmune diseases, viral infections,

chronic bacterial and parasite infections, and healthy individuals (in »5%). The positivity of RF in healthy individuals increases with age (2, 15, 16).
Antibodies against cyclic citrullinated peptide (CCP) are highly specific for RA (specificity 99%, sensitivity 59%). They predict an aggressive course of disease, and can be found in 15–30% of patients with negative RFs (15, 16).

Autoantibodies to neutrophil cytoplasmic antigens (ANCA) are targeted against different enzymes of azurophilic granules in neutrophils and lysosomes in monocytes. They are mainly used for diagnosing vasculitis of middle and minor arteries. Immunofluorescent methods are used for detecting specific immunofluorescence patterns. The two most important immunofluorescence patterns are cytoplasmic pattern (c-ANCA) and perinuclear pattern (p-ANCA). Anti-proteinase-3 (PR3) antibodies produce/stain c-ANCA fluorescence in 80–90% of cases and are typical of Wegener's granulomathosis. p-ANCA fluorescence patterns are produced in 50% of cases by anti-myeloperoxidase antibodies, which can be detected in microscopic polyangiitis and idiopathic necrotizing glomerulonephritis. ANCA titre changes with disease activity. Only c-ANCA antibodies have diagnostic value because of high specificity for Wegener's granulomathosis (4, 10, 17).

CONCLUSION

The pathogenesis and diagnosis of autoimmune diseases remains a subject of intense investigation and are thus complementary. The purpose of this article was to highlight some of the most common facts and draw attention (especially for non-internal medicine specialists) to these rare diseases because an early diagnosis is extremely important.

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