

Athanasios G. Tzioufas, **John G. Routsias** **Ιδιοτυπικό - αντιιδιοτυπικό δίκτυο αυτοαντισωμάτων: Παθογενετικές συσχετίσεις και κλινικές εφαρμογές.** *Autoimmunity Reviews* 9 (2010) 631–633

A common serologic finding in autoimmune diseases is the presence of autoantibodies against intracellular autoantigens. Recent data suggest that an anti-idiotypic network exists in these diseases, regulating the production of autoantibodies (idiotypic response). The anti-idiotypic antibodies can be monitored using complementary epitopes, designed according to the "molecular recognition" theory. The role of anti-idiotypic antibodies in neonatal lupus and type 1 diabetes are discussed. In neonatal lupus, mothers with high anti-idiotypic antibody activity against anti-La autoantibodies are at lower risk of giving birth to a healthy child, as compared with mothers without anti-idiotypic antibodies. Similarly, the lack of particular anti-idiotypic antibodies, against anti-GAD65 autoantibodies predispose in type 1 diabetes. These findings imply that anti-idiotypic antibodies may confer protection from the harmful effect of autoantibodies in certain autoimmune diseases.



Review

Idiotype, anti-idiotypic network of autoantibodies Pathogenetic considerations and clinical application

Athanasios G. Tzioufas*, John G. Routsias

Department of Pathophysiology, School of Medicine, University of Athens, 75, M. Asias st 11527 Athens, Greece

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ABSTRACT

A common serologic finding in autoimmune diseases is the presence of autoantibodies against intracellular autoantigens. Recent data suggest that an anti-idiotypic network exists in these diseases, regulating the production of autoantibodies (idiotypic response). The anti-idiotypic antibodies can be monitored using complementary epitopes, designed according to the “molecular recognition” theory. The role of anti-idiotypic antibodies in neonatal lupus and type 1 diabetes are discussed. In neonatal lupus, mothers with high anti-idiotypic antibody activity against anti-La autoantibodies are at lower risk of giving birth to a healthy child, as compared with mothers without anti-idiotypic antibodies. Similarly, the lack of particular anti-idiotypic antibodies, against anti-GAD65 autoantibodies predispose in type 1 diabetes. These findings imply that anti-idiotypic antibodies may confer protection from the harmful effect of autoantibodies in certain autoimmune diseases.

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1. Introduction

The regulation of the autoimmune response is still an intriguing and largely unexplored area. It is now appreciated that the autoimmune response is antigen-driven, since: (a) most of autoantibodies are of IgG class, pointing that an antigen-dependent T-cell help should be provided [1], (b) they are directed against multiple antigenic determinants (B-cell epitopes) within the autoantigen of which some are major epitopes, while some others are recognized by a minority of autoantibodies and considered as minor epitopes [1], (c) autoantibodies to particular epitopes possess high disease specificity and sensitivity, as well as an

almost perfect association with HLA class II antigens [2], (d) immunization of experimental animals with fragments of the autoantigens induces intra- and inter-molecular spreading of the immune response, similar to that observed after immunization with foreign antigens [3,4] and (e) extensive studies of the pathologic lesion in certain diseases, including rheumatoid arthritis, Sjögren's syndrome and thyroiditis Hashimoto revealed that the autoimmune lesion is organized in ectopic germinal centers, in which B lymphocytes may contain intra-cytoplasmic IgG with autoantibody reactivity, and abundance of autoantigen, available to prime the autoimmune response [5,6]. Following these observations, simple assumptions for the regulation of the autoimmune response can be made: adaptive autoimmune responses are regulated by two main factors: (a) those extrinsic to the immune system and particularly the autoantigen and (b) factors intrinsic to the immune system, including the local inflammatory milieu and internal regulatory mechanisms such as the idiotypic/anti-idiotypic network.

* Corresponding author. Tel.: +30 210 7462670; fax: +30 210 7462664.
E-mail address: agtzi@med.uoa.gr (A.G. Tzioufas).

2. Idiotypic and anti-idiotypic antibodies

The idiotypic network theory was proposed by the 1984 Nobel laureate Niels Jerne [7]. He hypothesized that antibodies can act as antigens and elicit anti-antibodies (called anti-idiotypic antibodies). In this regard an anti-idiotypic network is defined as regulator of the production of idiotypic antibodies contributing to the homeostasis of the adaptive immune response. Similarly, antibodies produced against an infectious agent can elicit anti-idiotypic antibodies that may have the incidental property of being antibodies to the host structure [8]. In general, anti-idiotypic antibodies can either “neutralize” the idiotypic antibodies or elicit, upon immunization, antibodies with the parental antigenic specificity. These functions are referred as the idiotypic–anti-idiotypic network serving as an intrinsic regulatory mechanism of the adaptive humoral immune responses. Recently, it has been shown that anti-idiotypic antibodies might also act as regulators of the autoimmune response in SLE [9]. Despite the attractive theory, presenting in the opening lines of this section, the detection of anti-idiotypic antibodies in clinical specimens is often challenging, since (a) autoimmune diseases present polyclonal responses to self antigen, as a result of the previously mentioned intra- and inter-molecular spreading, (b) some idiotypes are unique to each patient, and therefore the performance of general studies and assumptions is often difficult or even impossible and (c) the isolation of a homogenous population of antibodies is a difficult task due to cross-reactions generated by polyreactive antibodies [10].

The detailed knowledge of the antigenic structures within the autoantigen that is recognized by autoantibodies (B-cell epitopes), performed over the last decade led to the design of complementary epitopes, anticipated to be recognized by anti-idiotypic antibodies, as suggested by the “molecular recognition” theory [11]. According to this theory, a sense peptide, transcribed and translated from a nucleotide sequence read in the 5′→3′ direction binds to its complementary peptide counterpart, transcribed and translated in frame with that of its sense peptide from a nucleotide sequence read in the 5′→3′ direction on the opposite DNA strand. Previous experimental data, suggest that these interacting complementary peptides have the ability of generating and eventually detecting interacting pairs of idiotypic and anti-idiotypic antibodies [12].

3. Antibodies to complementary epitopes are anti-idiotypic antibodies to autoantibodies

Studies in our laboratory have demonstrated that in sera of patients with systemic lupus erythematosus (SLE) and Sjögren's syndrome, an active idiotypic–anti-idiotypic network exists, targeting the two major B-cell epitopes of La/SSB and their complementary peptides [13]. The anti-idiotypic antibodies were isolated using the complementary epitopes and found to bind anti-La/SSB antibodies, competing with La/SSB epitopes for their antigen binding site. In some cases the anti-idiotypic antibodies were capable of completely masking the anti-La/SSB antibodies, inhibiting their anti-La/SSB reactivity. A specific procedure was developed with the use of complementary peptides for the release of anti-La/SSB antibodies from their anti-idiotypic counterpart [13]. This procedure applied in anti-La (–), anti-Ro/ANA (+) sera from patients with SLE and primary Sjögren's syndrome. Ninety-four percent of pSS sera and 80% of SLE sera were found negative for anti-epitope 349–364 antibodies in ELISA prior to the treatment with complementary epitope. After unmasking the anti-La antibodies, all SS and SLE sera became positive for antibodies against the epitope 349–364aa, while none of the normal sera exhibited a positive reaction. Animal studies, also demonstrated that Balb/c mice immunized with complementary epitopes of La/SSB develop anti-human La/SSB antibodies [14], suggesting that the complementary epitopes of La/SSB have the potential of inducing an autoimmune response against La/SSB

autoantigen. Other findings indicate also that autoimmunity can be initiated through an immune response against a peptide that is complementary to the autoantigen. In fact, Pedergraft III and co-workers demonstrated that a subset of PR3-ANCA positive patients with necrotizing vasculitis has also antibodies directed against the translated protein product of the middle fragment (105–201 a.a.) of the antisense RNA of PR3, termed complementary PR3 or cPR3 [15]. These antibodies were not present in patients with vasculitis and anti-myeloperoxidase (MPO) autoantibodies (MPO-ANCA), patients with SLE, or healthy individuals. It was also demonstrated that human anti-cPR3 and anti-PR3 antibodies are in fact an idiotypic–anti-idiotypic pair, since mice immunized with cPR3 develop both anti-cPR3 and anti-human PR3 antibodies while complementary PR3 transcripts are present in the peripheral leukocyte RNA from a subset of ANCA patients [15,16].

4. Anti-idiotypic antibodies and human disease. The examples of NLS and type1 diabetes

Neonatal lupus syndrome (NLS) is considered as the exemplary model of passively acquired systemic autoimmune disease. Maternal IgG autoantibodies against Ro/SSA and/or La/SSB are transported through the placenta and harm the fetus by causing injury to the skin and heart. NLS is characterized by two dominant manifestations, cutaneous rash and congenital heart block (CHB), the latter being most often of third-degree severity in a structurally normal heart [17]. The true incidence of NLS is unknown [18], but CHB is estimated to occur in about 2% of anti-Ro/SSA-positive mothers [19]. The presence of anti-La/SSB antibodies may increase the risk of CHB in the fetus to 5% as compared with the presence of anti-Ro/SSA alone [20]. A putative role for the candidate antibodies in the pathogenesis of this disease derives from *in vitro* and *in vivo* data, demonstrating that maternal anti-Ro/SSA and/or anti-La/SSB antibodies opsonize fetal apoptotic cardiomyocytes, which in turn induces a proinflammatory/profibrotic response by phagocytosing macrophages, ultimately leading to tissue injury [21]. Since, complementary peptides have the potential to adopt structures that are complementary to B-cell epitopes and mimic the shape of the paratopes of the antibodies recognizing these epitopes, they can be efficiently used for the detection of anti-idiotypic antibodies [22]. Among the systemic autoimmune diseases, NLS is the ideal model for studying anti-idiotypic antibodies, since pathogenetic autoantibodies to Ro/SSA and/or La/SSB are directly involved in tissue injury [23].

In a recent work of our laboratory, the idiotypic/anti-idiotypic network of antibodies targeting the dominant epitopes of La/SSB in mothers positive for anti-Ro and/or anti-La/SSB antibodies were evaluated, in an attempt to define the role of this network in the development of NLS [24]. To accomplish this task, peptides and complementary peptides deduced from the sequences 289–308aa and 349–364aa of La/SSB (shown previously to be the major B-cell epitopes of La/SSB [25]) were synthesized and tested against maternal sera. It was found that sera from mothers giving birth to a healthy child and having no history of a child with NLS exhibited higher anti-idiotypic antibody activity compared to mothers carrying a child with NLS or mothers giving birth to a healthy child but who previously gave birth to a child with NLS. Sera from mothers of healthy children, which exhibited no apparent epitope activity against amino acids 349–364, revealed a significantly higher frequency of hidden anti-349–364aa epitope responses, blocked by anti-idiotypic antibodies, as compared to sera from women pregnant with an affected child [24]. Therefore, the presence of anti-idiotypic antibodies to autoantibodies against La/SSB may protect the fetus by blocking pathogenic maternal autoantibodies (Fig. 1). Testing for these anti-idiotypic antibodies may be useful as a predicting factor of low risk pregnancies for NLS.

Type 1 diabetes (T1D) is an autoimmune disease characterized by the presence of autoantibodies to multiple islet cell autoantigens.

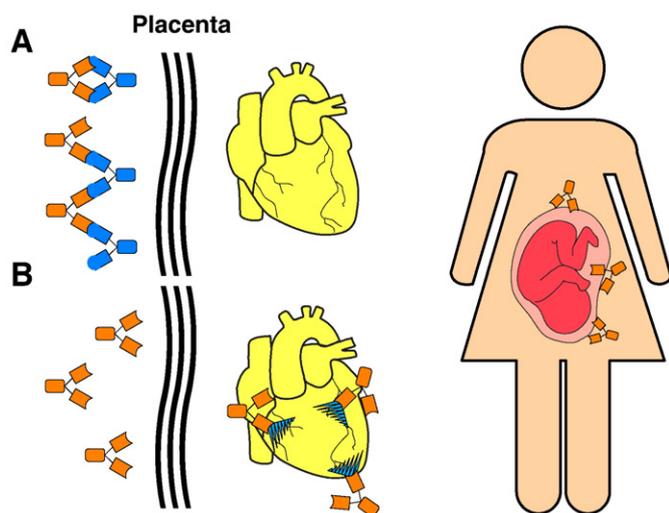


Fig. 1. (A) In neonatal lupus, the presence of anti-idiotypic antibodies to autoantibodies against La/SSB may protect the fetus by blocking pathogenic maternal autoantibodies. In this regard, large immune complexes are formed preventing cross of pathogenic autoantibodies through the placenta. (B) If anti-Id antibodies do not exist, maternal anti-La IgG autoantibodies pass through the placenta to the fetal circulation and cause tissue injury to the developing heart.

Autoantibodies to glutamate decarboxylase 65 (GAD65Ab) are detected in the majority of new-onset T1D patients [26], in adult patients with latent autoimmune diabetes [27] and in some rare neurologic diseases such as Stiff Person Syndrome (SPS) [28], but rarely in the general population. The presence of GAD65Ab often herald the onset of T1D by months or even years and are used to predict disease together with other autoantibodies to islet cell [29]. In a recent work, Oak et al demonstrated that masked GAD65Ab are present in the healthy population and that a lack of particular anti-Ids, rather than GAD65Ab *per se*, is a characteristic of T1D [30]. Therefore, anti-Ids may play a protective role in the immune response, by preventing GAD65Ab to bind to their antigen and potentially modulate T-cell responses to GAD65.

Take-home messages

- Intracellular autoantigens contain multiple B-cell epitopes, useful to study the regulation of the autoimmune response.
- Sera of autoimmune patients contain an active idiotypic–anti-idiotypic network, which can be induced also in experimental animals, following immunization with B-cell epitopes of autoantigens.
- Anti-idiotypic antibodies are targeted using complementary epitopes, corresponding to major B-cell epitopes.
- Sera of pregnant women with anti-La/SSB autoantibodies who carry a healthy baby, have significantly higher levels of anti-idiotypic antibodies to anti-La/SSB, suggesting that these may serve as protecting antibodies for the development of CHB.

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