Natural Hidden Autoantibodies

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Key words: natural autoantibodies, autoimmunity, anti-DNA, anticardiolipin, hidden autoantibodies

IMAJ 2007;9:748–749

Autoimmune diseases are present in about 3% of the general population. Almost all of the human autoimmune diseases are characterized by the generation of autoantibodies. Identifying those autoantibodies is the cornerstone for the diagnosis of autoimmunity in humans. In autoimmune rheumatic diseases, pathogenic autoantibodies are used for classification, development of diagnostic criteria, monitoring of disease activity and prediction of prognosis. However, autoimmunity defined by the detection of autoantibodies does not necessarily imply the presence of an autoimmune disease. Furthermore, the normal immune system is able to produce, in relatively high amounts, antibodies that bind various self-antigens. Those autoantibodies, defined as natural autoantibodies, have an important physiological regulatory role [1].

Differentiating between pathogenic, natural and other non-pathogenic autoantibodies is crucial for the definition, diagnosis and identification of a reliable biomarker of autoimmune diseases [2]. The identification of autoantibodies that highly predict the development of autoimmune diseases is of great interest.

Natural antibodies are produced mainly by CD5-positive B cells. They belong mainly to the immunoglobulin M class and are characterized by several features, including the ability to bind self and non-self antigens, low affinity (monovalent antigenic binding to a small single epitope), high avidity (overall force that binds multivalent antibody to a macromolecule carrying multivalent epitopes), and polyreactivity (binding different epitopes). In addition, the natural autoantibodies are interconnected in the idiotypic network. The ability of natural autoantibodies to react with corresponding antigens and to induce a biological reaction is determined by the titer of the autoantibody, concentration of antigens, and affinity and avidity characteristics.

Pathogenic autoantibodies are antigen driven, produced by CD5-negative cells and belong mainly to the IgG isotype. Unlike natural autoantibodies, they bind a single epitope (monoreactive) with very high affinity [3]. Pathogenic autoantibodies are generated years before the development of clinical symptoms of the autoimmune disease, and the detection of those autoantibodies in the sera of asymptomatic individuals may be used for screening for certain autoimmune diseases [2].

Fourteen years ago, Shoenfeld and co-workers [4,5] reported that normal human immunoglobulins, eluted from protein A chromatography and anti-human IgG chromatography columns, reacted with cardiolipin, DNA, phosphatidylserine, phosphatidylinositol, thyroglobulin and other self-antigens. However, they found that the original intact normal human sera had no autoantibody activity against all of the tested autoantigens. In addition, the eluted immunoglobulins lost their ability to react with the autoantigens by mixing them back with the corresponding original sera. Those autoantibodies that cannot be detected by the conventional methods used to screen sera are termed "hidden" autoantibodies [6].

Hidden autoantibodies were also identified in immunoglobulin fractions eluted, from sera of healthy individuals, by various anti-immunoglobulin or protein A columns using acid-gel filtration on Sephadex G-200 [7], affinity chromatography [8], and immunoadsorption on protein G agarose [9]. The eluted immunoglobulins reacted with cardiolipin, dsDNA, pyruvate dyhyrogenase, histones, Fc fraction of immunoglobulins (rheumatoid factor), ribosomal P and others [4-9].

It has been suggested that hidden autoantibodies have all the characteristics of natural autoantibodies. They are generated by the normal immune system by CD5-positive B cells and, similar to other natural autoantibodies, possibly constitute a physiological repertoire that may have a regulatory role [6].

Hidden autoantibodies bind their autoantigens specifically. The binding of a hidden anticardiolipin to cardiolipin was inhibited by pre-incubation of the eluted IgG immunoglobulins with various concentrations of cardiolipin and other phospholipids. However, the binding of hidden anticardiolipin to various phospholipids was competitively inhibited by adding increasing concentrations of normal sera. This observation suggests that normal sera had

IG = immunoglobulin

natural inhibitors that prevent the specific binding of hidden normal autoantibodies to their autoantigens in whole sera [4,5].

Studies on pathogenic autoantibodies failed to support these findings. The binding of pathogenic anticardiolipin antibodies derived from patients with antiphospholipid syndrome to cardiolipin was inhibited by increasing concentration of cardiolipin, but adding normal sera did not decrease its binding to cardiolipin [5].

Hidden autoantibodies were also detected in whole sera by changing their physiological characteristics. Hidden antiphospholipid antibodies were detected by heating the sera for 30 minutes at 56°C [10]. In addition, hidden autoantibodies were detected in normal human sera by adding acid pH, hyermolar buffers or by phospholipase digestion of normal sera [10].

Recently, McIntyre et al. [11] described a new class of hidden autoantibodies that are not detected by conventional screening methods. Autoantibody activity was identified in the blood of healthy donors after being cultured overnight in blood culture bottles. They suggest that changing the physiological features of the sera by adding hemin, which contains iron, results in the emergence of autoantibody activity in normal sera. Antiphospholipid activity was identified by adding purified hemin to IgG, and this activity was directly inhibited by adding normal serum. They postulate that hemin changes the physiological characteristics of IgG by an oxidation-reduction interaction. Therefore, the authors suggest terming those natural hidden autoantibodies "redox" (reduction-oxidation) autoantibodies. In addition to antiphospholipid antibodies, a wide range of autoantibodies was detected by the redox reaction, including antimyeloperoxidase, antinuclear antibodies, antimitochondrial and other autoantibodies. The data presented in that paper indicate that the "redox" autoantibodies represent a group of hidden natural autoantibodies. The authors did not describe the basic characteristics of their autoantibodies, including isotype, affinity, avidity and polyreactivity features. Therefore, "redox" cannot be classified as a new class of autoantibodies. Like other investigators, they report a new method of unmasking the autoantibody activity of hidden autoantibodies.

In summary, the presence of hidden autoantibodies in the sera of healthy people is established [5]. They are detected by separating the immunoglobulin from the whole serum, heating or adding various chemical components that alter the physiological features of the immunoglobulin. It is plausible that exposure to environmental antigens leads to a change in the physiological characteristics of the blood and unmasks the autoantibody activity of hidden autoantibodies.

Hidden autoantibodies bind a wide range of self-antigens; however, they are not pathogenic and cannot be used in screening strategies for early diagnosis of autoimmune diseases. Solid evidence supports a role for natural autoantibodies in biological and physiological regulation.

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