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The natural autoantibody repertoire and autoimmune disease

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Abstract

The incidence of autoimmune diseases has shown a significant increase in developed countries during the last 40 years. The cause of this increase is still unknown, and reliable methods for the detection of individuals at risk of developing autoimmune disease are not available yet. To explore new methods for the diagnosis and monitoring of autoimmune disease, we have studied the repertoire of natural autoantibodies (NA) and its relationship with autoimmune disease using large arrays of defined antigens. NA are found in healthy humans and mice, apparently in the absence of immunization with their target antigens. We used knock-out mice to demonstrate that the repertoire of NA is influenced by factors not directly related to antigenic stimulation such as endogenous levels of histamine. By studying strains of mice known to differ in their susceptibility to autoimmune disease, we could conclude that the repertoire of NA reflects the susceptibility to develop autoimmune disease. The study of the human repertoire of NA required the development of bio-informatic tools to overcome the variation introduced by individual differences in the genetic background and immune history. We found that human NA are organized in clusters that can differentiate healthy subjects from patients with type 1 diabetes mellitus, type 2 diabetes mellitus or Behçet's disease patients. The development of new tools to undertake large-scale NA analysis could also enhance our understanding of the immune system, and leave us in a better position to face the up-coming epidemics of autoimmune disorders.

Keywords: Natural autoantibodies; Autoimmunity; Protein microarrays

1. Natural autoantibodies

A healthy immune system contains self-reactive B- and T-cells [19,20,28,34,36,40]. The antibodies reactive with self or foreign molecules detectable in the absence of known immunization with the target antigen are termed natural antibodies (see [5,15,36] for a comprehensive review on the subject). Here, we focus on the natural autoantibodies (NA) detectable in the repertoire of serum immunoglobulins. NA can show T-cell independent isotypes, such as IgM, but also T-cell dependent isotypes, like IgA and IgG, suggesting that self-reactive T-cells can support the generation of NA [5,15,36,58]. Therefore, the repertoire of NA might be used to study both B- and T-cell autoreactivity.

1.1. Origin and function of natural antibodies

NA seem to be conserved through evolution [5,15] suggesting that they are not simple side-products of exogenous immunization, they might have a physiological role in body homeostasis. Indeed, natural autoimmunity performs several functions that include the scavenging of metabolic waste and senescent cells [25], a first line of protection against viral and bacterial infection [45,46], the control of autoimmune diseases [3,12,30,37] and the repair of the central nervous system following trauma [13].

A role for NA in body homeostasis is reinforced by the finding that NA are genetically controlled and apparently independent of environmental antigen stimulation [24,27,30,42,43,61]. It has been estimated that 5–15% of splenic B-cells activated in vivo can secrete NA [29]. However, the mechanism of activation of self-reactive B-cells is still a matter of debate, and possible candidates are anti-idiotypic interactions [37] together with the direct recognition of autoantigens [28].

1.2. Natural autoantibodies and autoimmune disease

NA can recognize self-antigens that are also targeted during the progression of autoimmune disorders; these antigens include insulin, DNA, myelin basic protein and thyroglobu-

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lin among others [10–12]. After reviewing the literature describing NA in humans, Lacroix-Desmazes et al. [36] concluded that the NA present in healthy individuals are indistinguishable from the autoantibodies found in autoimmune disease in terms of V gene usage, extent of mutations, affinity and specific reactivity. Nevertheless, NA and disease-associated autoantibodies differ in their quantity and fine epitope specificity [7,36].

The detection of NA in healthy individuals is in line with the idea of the immunological homunculus [9,14]. The concept of the immunological homunculus is based on the observation that natural T- and B-cell autoimmunity is usually organized around a particular set of self-antigens [9,14]. Therefore, autoimmune disorders might simply arise as a consequence of the dysregulation of natural, physiological, autoimmunity. Indeed, it has been proposed that NA can give rise to pathogenic autoantibodies [36]. The mechanisms involved in the shift from physiological to pathological autoimmunity are unknown. One plausible mechanism is the induction of an immune response directed against a self-antigen that has undergone physicochemical alterations or is abnormally presented to autoreactive T-cells [36]. Alternatively, pathogenic autoantibodies might arise as a consequence of an environmental trigger [36] or the removal or absence of immuno-regulatory mechanisms due to genetic deficiencies [36].

An immune system harboring self-reactive clones that can switch to pathogenic clones is potentially dangerous, and so regulatory mechanisms have evolved to keep them under control. Regulatory mechanisms based on anti-idiotypic interactions [30,37] and regulatory cells [6,39,54,55] have been described. In addition, natural IgM autoantibodies might also participate in immune regulation. IgM deficient mice have higher levels of circulating IgG autoantibodies [18]. Moreover, natural IgG autoantibodies purified from sera (and therefore relieved from their interaction with regulatory IgM) show a greater degree of self-reactivity than IgG antibodies in the original, unfractioned, serum ([41] and F.J. Quintana and I.R. Cohen, unpublished observations). The differences in antibody reactivity existing between unfractioned and purified IgG are not detectable in serum samples from autoimmune disease patients [30]. Taken together, these results suggest that IgM NA can control the autoreactivity of IgG NA in serum; indeed, some regulatory actions of serum IgM have been shown to be mediated through anti-idiotypic interactions [30,37].

1.3. The study of the repertoire of natural antibodies

Coutinho and his colleagues pioneered the study of the repertoire of NA by analyzing the binding of whole serum or purified antibodies in a quantitative western blot [26,44,58]. Their studies used tissue extracts as a source of self- or non-self-antigens, analyzing the patterns of antibody reactivity using principal component analysis alone or in combination with hierarchical clustering [26,44,58]. Although this method has been helpful for the study of the general organization of the repertoire of NA, the nature of the antigens used (complex mixtures of self- or foreign-antigens) impedes the easy identification of the targets recognized by NA. Thus, this methodology is not the most appropriate for the study of the connection between defined NA and autoimmune disease.

We have studied the repertoire of NA using an unbiased solid-phase ELISA antibody test designed to detect even small amounts of low affinity serum IgG and IgM antibodies binding to an array of almost 100 different antigens, mostly self-antigens [22,23,50–53]. We did not intend to replace traditional approaches to immune diagnosis; however, instead of only identifying specific single autoantibodies, our analysis also aimed to study *patterns* of autoantibodies. To accomplish this goal we adapted bio-informatic tools initially developed for analyzing data from gene arrays, and used them to investigate the organization of NA and to make diagnostic classifications [22,23,52].

2. The repertoire of autoantibodies in the mouse

2.1. Association of NA with susceptibility to autoimmune disease

We examined the association between NA and autoimmune disease in serum samples of naive mice of strains known to differ in their susceptibility to spontaneous or induced autoimmune disease [51]. Non-obese diabetic (NOD) mice spontaneously develop autoimmune diabetes, while C57BL/6 mice are relatively resistant to autoimmune disease [16]. However, several experimental autoimmune diseases can be induced in NOD and C57BL/6 mice. NOD mice are susceptible to the induction of systemic lupus erythematosus (SLE) and experimental autoimmune encephalomyelitis (EAE) [35,57]; while SLE, EAE and experimental autoimmune myasthenia gravis (EAMG) can be induced in C57BL/6 mice [4,35,57].

IgM and IgG autoantibodies detected in the sera of NOD and C57BL/6 mice showed different patterns of reactivity [51]. Naïve NOD mice harbored IgG NA directed not only against self-antigens associated with autoimmune diabetes, they also manifested antibodies associated with the experimental autoimmune diseases to which they are susceptible: SLE and EAE [51]. Conversely, naïve C57BL/6 mice manifested IgG NA antibodies to the self-antigens associated with the diseases to which they are susceptible: EAE, SLE and EAMG [51]. These results suggested that the identity of naturally occurring IgG autoantibodies could reflect the susceptibility to the induction of specific autoimmune diseases.

The IgM autoantibodies in C57BL/6 serum reacted with more self-antigens and showed higher OD values than did the IgM autoantibodies from NOD mice [51], reinforcing the idea that IgM autoantibodies may be associated with mechanisms of immunoregulation. Indeed, knock-out mice unable

to produce IgM antibodies are more prone to harbor autoantibodies than their wild-type (WT) counterparts [18].

2.2. Experimental autoimmune myasthenia gravis in the NOD mouse: a test case

We observed that NOD mice spontaneously produce IgG antibodies to the acetylcholine receptor [51], a protein targeted by pathogenic antibodies in EAMG in susceptible rodents [8]. However, at the time, there were no reports describing that NOD mice were indeed susceptible to EAMG induction. To test whether the presence of specific spontaneous IgG autoantibodies can predict susceptibility to an autoimmune disease, we challenged NOD mice with a standard protocol used to induce EAMG [53]. The NOD mice developed EAMG, although to a somewhat lesser degree than did C57BL/6 mice, a strain regarded as highly susceptible to the disease [53]. These results support the association between specific IgG NA and susceptibility to a particular autoimmune disease.

2.3. Endogenous levels of histamine influence the repertoire of autoantibodies

Histamine has been shown to influence many aspects of the immune response, including antibody production [32,33,56]. We have therefore characterized the repertoire of NA in histidine decarboxylase knock-out (HDC-KO) mice [47], unable to synthesize histamine.

HDC-KO and WT mice differed in the patterns of reactivity of their IgM and IgG NA [50]. The NA in HDC-KO sera manifested a larger repertoire of IgM autoantibodies than did the WT sera [50]. The self-antigens bound by IgM from HDC-KO mice included structural proteins, enzymes associated with cellular metabolism, double-stranded and single-stranded DNA, and tissue-specific antigens like insulin [50]. There were relatively fewer differences in the NA repertoire of IgG autoantibodies of the mice: notably, the HDC-KO sera reacted with glutamic acid decarboxylase [50], an antigen associated with autoimmune diabetes [59]. We are currently checking whether GAD-specific antibodies in HDC-KO mice reflect an increased susceptibility to develop autoimmune diabetes. Nevertheless, our results demonstrate that factors not directly related to antigenic stimulation such as endogenous levels of histamine can influence the NA repertoire. Thus, immune disorders characterized by altered levels of endogenous histamine, such as allergies, might be reflected as specific alterations in the repertoire of NA.

3. The repertoire of natural antibodies in humans

Unlike the relatively few and well defined IgG autoantibodies present in naïve pathogen-free mice, healthy humans manifest IgG NA to many antigens [52]. In addition, each individual human has its own genetic background and immune history, rendering simple NA repertoire comparisons unfeasible. We applied bio-informatics to analyze patterns of IgM and IgG autoantibodies in the sera of 20 healthy persons and 20 persons with type 1 diabetes mellitus (T1DM) [52]. The healthy subjects manifested autoantibodies to a variety of self-antigens, many known to be associated with autoimmune diseases [52]. Using a coupled two-way clustering algorithm developed for analyzing data from gene arrays [21] we studied the organization of the NA in the samples of healthy subjects of T1DM patients. The reactivity patterns of NA to particular subsets of self-antigens were organized and could significantly discriminate between healthy persons and T1DM patients [52]. Thus, although NA are highly prevalent in humans, the study of their patterns of reactivity can provide information about the state of the body and generate diagnostic classifications.

We have recently extended our analysis of human NA to include samples taken from patients affected by type 2 diabetes mellitus (T2DM) and Behçet's disease (BD) [22,23]. The patterns of NA could separate T2DM samples from those taken from healthy donors, T1DM or BD patients [22,23]. Although the finding of specific autoantibodies in T2DM had been already described [60], our study appears to be the first to analyze the NA repertoire in patients affected by this metabolic disorder. Overall, these results suggest that the analysis of patterns of human NA might have diagnostic applications. In addition, our results suggest that non-autoimmune disorders too can be reflected as alterations in the organization of the repertoire of NA. Finally, large-scale prospective studies are needed to analyze the power of techniques based on the analysis of patterns of NA for the detection of individuals at risk of developing a specific autoimmune disease.

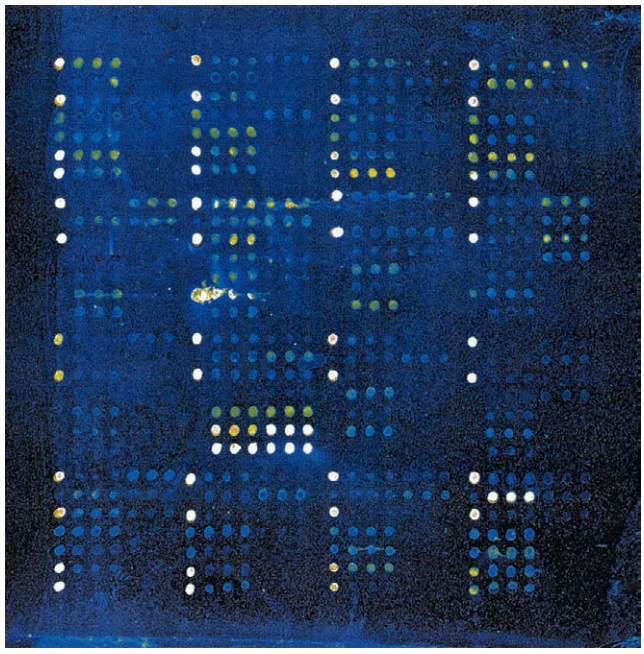

Fig. 1. A representative antigen chip showing the IgG reactivity of a test sample against an array of 224 different antigens.

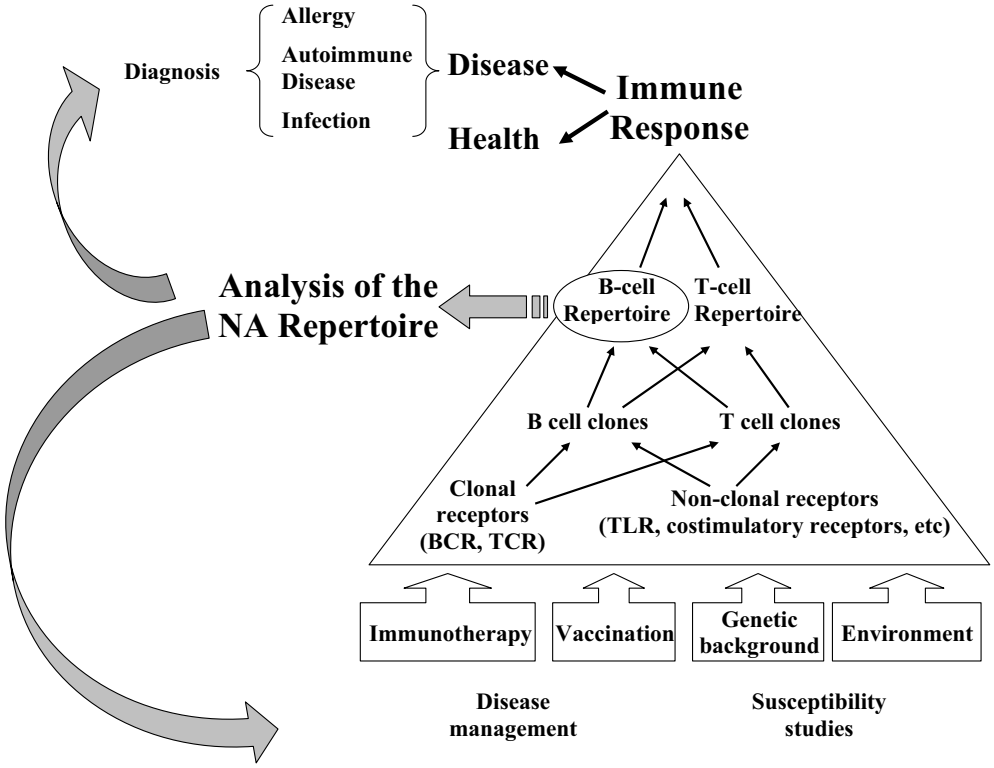

Fig. 2. Schematic representation of the applications that the analysis of the NA repertoire might have on the prevention, monitoring and management of human autoimmune diseases.

4. General remarks and future directions

The clinical use of NA repertoire analysis would be facilitated by the use of protein microarrays to analyze hundreds of NA in parallel, using only small amounts of serum samples and antigens. The construction of these microarrays has been recently reported [38]; however in their present format they were applied to detect high-titer antibodies, but they are not suitable for the study of NA. However, we have succeeded in developing antigen microarrays suitable for the analysis of NA repertoires, and we are presently checking their performance on human and murine samples (Fig. 1). The profiling of the repertoire of natural antibodies with protein arrays might expedite the diagnosis and therapy of immune disorders, allowing not only the rapid diagnosis after the onset of the disease, but also the early detection of individuals susceptible to develop immune disorders and the adjustment of the immunotherapy according to the individual needs of the patient (Fig. 2).

In recent years, complex systems biology has changed the way we look at biological processes such as gene expression [31]. The complex systems biology approach is based on the study of the behavior of complete biological systems and the hundreds of interactions that characterize them [2,48], and not in the isolated behavior of its components. Immunological research has been traditionally focused on the study of selected antigens associated with specific autoimmune diseases [1,49]. However, the existence of natural autoimmunity and its physiological functions [36] hampers efforts aimed at establishing one-to-one relationships between particular B- or T-cell clones and a particular disease. Within this scenario, immunology will certainly benefit from the use of a complex systems approach for the analysis of immune system behavior. Antigen chips might therefore became central tools for the collection of data reflecting the response of the immune system when faced with challenges such as vaccination, infection or autoimmunity. These data-collecting devices, together with the use of bio-informatic tools that allow us to integrate and visualize the data in a simple but informative way [17], will lead to a deeper understanding of immune function and immune-related disorders. Then, the challenge will be to translate the new knowledge about the organization of the immune system into safer and more effective immunotherapies.

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