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Biological Functions of Catalytic Antibodies

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Key Words
Catalytic antibodies · Autoimmune diseases · Hemophilia A · Idiotypic network · Factor VIII

Summary
The extent of the repertoire of antibodies that may be potentially produced by the organism is determined by the number of V_H, D_H, J_H, V_L and J_L genes that encode the variable regions of immunoglobulins and by the junctional diversity that occurs at the time of somatic rearrangement. This potential repertoire includes antibodies the antigen binding site of which may recognize external as well as autologous antigens (the later being referred to as natural antibodies), or may structurally resemble the active site of enzymes and be endowed with enzymatic activity. Under physiological conditions, B-cell clones that produce antibodies naturally endowed with catalytic activity are negatively regulated and subjected to apoptosis. Catalytic antibodies are expressed only following active immunization of the organism using haptens that are analogs of transitions states for chemical reactions, or if the physiological regulatory mechanisms that control the expression of catalytic antibody-producing B-cell clones are perturbed, e.g. in the context of pregnancy or in the course of autoimmune diseases.

Schlüsselwörter
Katalytische Antikörper · Autoimmunerkrankungen · Hämophilie A · Idiotypisches Netzwerk · Faktor VIII

Zusammenfassung
Das in einem Organismus theoretisch verfügbare Antikörper-Repertoire ergibt sich aus der Anzahl der V_H-, D_H-, J_H-, V_L- und J_L-Gene, die die variablen Regionen der Immunglobuline kodieren, und aus der Generierung der junktionalen Vielfalt während des Prozesses der somatischen Rekombination. Dieses theoretisch verfügbare Antikörper-Repertoire schließt mit Fremdantigen und mit autologem Antigen reaktive Antikörper (natürliche Antikörper) ebenso ein wie Antikörper, die strukturelle Ähnlichkeit mit dem aktiven Zentrum von Enzymen haben und mit enzymatischer Aktivität ausgestattet sein können. Unter physiologischen Bedingungen unterliegen B-Zell-Klone, die natürliche Antikörper mit katalytischer Aktivität produzieren, einer negativen Regulation und in der Folge dem Eliminationsmechanismus der Apoptose. Katalytische Antikörper können unter zwei Voraussetzungen exprimiert werden: 1) Aktive Immunisierung des Organismus; das zur Immunisierung führende Hapten stellt ein Übergangszustand-Analogon (transition-state analog) einer chemischen Reaktion dar; 2) Versagen der physiologischen regulatorischen Mechanismen, die katalytische Antikörper produzierende B-Zell-Klone unterdrücken, z.B. im Rahmen einer Schwangerschaft oder im Kontext von Autoimmunerkrankungen.

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Introduction

The concept that some antibodies may by endowed with catalytic activity was first proposed by Linus Pauling in the early 1940s [1]. If, according to Pauling, the structure of the antigen binding site of antibodies were to be produced in a random manner, the antigen binding site of some of the antibodies may resemble the active site of enzymes and the latter antibodies may express enzymatic activity. It was not until the development the hybridoma technology by Köhler and Milstein in 1975 allowing to produce antigen-specific monoclonal antibodies that the hypothesis raised by Pauling was confirmed. The first catalytic antibodies were reported in 1986. Several approaches have been developed to raise catalytic antibodies; they all depend on the active immunization of an organism using haptens or protein antigens and are discussed below. The presence of catalytic antibodies under physiological and pathological conditions in humans without deliberate immunization is also discussed.

Production of Catalytic Antibodies by Immunization with Haptens

The large majority of catalytic antibodies has been produced following immunization to haptens that are stable transition-state analogs of chemical reactions. In 1986, Tramontano et al. [2] has raised a monoclonal antibody able to hydrolyze the ester bond by using an hapten that was an analog of the transition state for the hydrolysis of carboxylic esters. In the same year, Pollack et al. [3] produced a murine monoclonal antibody with high affinity to the p-nitrophenylphosphorylcholine transition-state analog. This antibody was able to catalyze the hydrolysis of the carbonate bond. Two alternative approaches have been developed since then: Reactive immunization is based on haptens that are so highly reactive that a chemical reaction occurs in the antibody combining site during immunization [4]; the 'bait and switch' technique implies haptens that contain structures complementary to the residues that are wished for in the antigen binding site of the catalytic antibody, for instance electric charges [5]. These strategies, that essentially rely on the chemical synthesis of haptens, have allowed the generation of antibodies that catalyze reactions such as electrophil addition/elimination, racemization, isomerization, hydrolysis, and formation of carbon-carbon bonds [6, 7]. One major aim of using transition-state analogs is the possibility to raise antibodies that catalyze thermodynamically or kinetically unfavorable reactions which can not be catalyzed by naturally occurring enzymes or by the chemical methods available to date [8].

Generation of Catalytic Antibodies or Abzymes by Manipulation of the Idiotypic Network

An alternative and more 'biological' approach that was used with success to raise catalytic antibodies relies on the concept of the 'internal image' that is constitutive to the idiotypic network and that was initially proposed by Niels Jerne in 1974. Animals are immunized using an enzyme in order to produce a monoclonal antibody (referred to as Ab1) the antigen binding site of which is structurally complementary to the active site of the enzyme. Monoclonal antibodies (Ab2) specific for the antigen binding site of the Ab1 are generated. Some of these Ab2 will bear a structural image of the active site of the enzyme and will mimic the catalytic function of the enzyme. This original approach has allowed the production of antibodies endowed with esterase activity [9], amidase activity [10] and serine protease activity [11], by using acetylcholine esterase, β-lactamase and subtilisin as immunogens, respectively. The kinetic parameters of the catalytic antibodies are the V_{max} (maximal rate of catalysis at saturating concentration of substrate), the K_m (affinity for the substrate), the K_{cat} (number of cycles of catalysis per unit of time) and the catalytic efficiency (expressed as the number of moles of substrate catalyzed per mole of enzyme and per unit of time). The kinetic parameters of anti-idiotypic abzymes are lower than that of the original enzymes, but higher than that of catalytic antibodies raised using transition-state analogs.

Catalytic Antibodies in Human

Catalytic antibodies may also be generated spontaneously by the immune system, without deliberate immunization. It has been proposed that such 'natural' catalytic antibodies may participate directly to the elimination of the biochemical wastes that are released by the metabolism of the organism under physiological conditions [12]. Furthermore, the presence of catalytic antibodies endowed with protein kinase and DNase activity in human milk suggests a protective role for catalytic antibodies in physiology [13, 14]. However, the majority of the studies performed in human being have demonstrated that the prevalence of catalytic antibodies increases under pathological conditions and, in particular, in the context of autoimmune disorders. The first catalytic antibodies described were isolated from patients with asthma and were able to cleave the vasoactive intestinal peptide [15]. Since then, proteolytic antibodies specific for thyroglobulin, prothrombin and factor VIII (FVIII) have been reported in patients with thyroiditis, multiple myeloma, or hemophilia A, respectively [16–18]. DNA- and RNA-hydrolyzing antibodies have been isolated from the serum of patients with different systemic autoimmune manifestations: systemic lupus erythematosus, sclerodermia, rheumatoid arthritis or multiple sclerosis [19–21]. It is in general intricate to determine whether or

not the catalytic antibodies found in patients play a role in the etiology of the disease and whether or not their occurrence is a result of the disease or is responsible for the scored clinical manifestations. However, in the case of patients with hemophilia A, hydrolysis of FVIII by proteolytic antibodies may be directly relevant to the inactivation of infused FVIII and contribute to the worsening of the deficit in hemostasis [22].

Factor VIII-Hydrolyzing Antibodies in Hemophilia A

Hemophilia A is an X chromosome-linked recessive hemorrhagic disorder characterized by impaired production of FVIII. Anti-FVIII IgG antibodies arise in 25–50% of patients with hemophilia A following therapeutic administration of exogenous FVIII. FVIII inhibitors have been shown to neutralize FVIII activity by steric hindrance. The occurrence of FVIII inhibitors is one of the major complicating factors in the treatment of hemophilia A, precluding the further use of therapeutic FVIII. Because steric hindrance of the interaction of FVIII with other coagulation factors by patients' IgG does not systematically account for the kinetics of FVIII neutralization, we wondered whether some anti-FVIII IgG may hydrolyze FVIII.

Plasma from hemophilia A patients with FVIII inhibitors were collected. Anti-FVIII antibodies were purified by affinity chromatography on protein G and size exclusion chromatography in denaturing conditions. Incubation of biotin-labeled FVIII with purified IgG resulted in FVIII hydrolysis, as demonstrated by Western blotting. Interestingly, IgG did not cleave human serum albumin or factor IX, thus providing indirect evidence that the catalytic activity of these antibodies is solely directed towards FVIII. In our latest study [22], significant hydrolytic activity was detected in the IgGs of 13 of 24 patients, giving a prevalence of catalytic FVIII inhibitor antibodies of 54%. A significant and positive correlation was seen between the extent of FVIII hydrolysis and the degree of FVIII inhibitory activity in these patients. Thus, the findings suggest that hydrolysis of FVIII by IgG plays a major role in the inhibition of FVIII activity in some patients.

Several lines of evidence confirm that FVIII hydrolysis is mediated by antibodies and exclude the possibility of a contamination with proteases [18, 22]:

- the catalytic activity to FVIII is co-eluted with anti-FVIII IgG;
- removal of IgG from affinity-purified anti-FVIII antibodies results in a complete loss of the hydrolyzing capacity;
- co-incubation of FVIII and anti-FVIII IgG in the presence of several protease inhibitors (E-64, pepstatine, leupeptine) does not prevent FVIII hydrolysis;
- size exclusion chromatography of urea-treated anti-FVIII IgG yields a major peak that is devoid of contaminants and retains the catalytic activity to FVIII;
- anti-FVIII IgG from different patients exhibit different kinetics of FVIII hydrolysis and different digestion patterns, suggesting that FVIII hydrolysis is mediated by the variable regions of antibodies;
- $F(ab')_2$ fragments of affinity-purified anti-FVIII IgG are able to cleave FVIII, further suggesting that FVIII hydrolysis is mediated by the variable regions of the antibodies.

The finding that a significant proportion of IgG isolated from hemophilia A patients with FVIII inhibitors may have hydrolytic activity is the first example of catalytic antibodies presenting with a putative role in the etiology of a disease. Potential pathological effects of these proteolytic anti-FVIII IgG include direct hydrolysis-mediated inactivation of FVIII or the indirect effect of presentation of new epitopes by antigen-presenting cells. In patients without catalytic FVIII inhibitors, FVIII is taken up by antigen-presenting cells through the Fc receptors and cleaved in by the endosomes. The resultant immunodominant peptides may be presented on the MHC. If FVIII is cleaved before encountering the antigen-presenting cells, it is possible that not all of the resulting peptides will be taken up by the antigen-presenting cells, resulting in different peptides being presented on the MHC. This would have the effect of skewing the anti-FVIII immune response towards new FVIII epitopes.

Bactericidal Activity of Antibodies

Wentworth et al. [23] recently demonstrated that all antibodies are potentially endowed with bactericidal activity. Indeed, antibodies are intrinsically able to promote the production of hydrogen peroxide (H_20_2) and ozone. This property is conserved among antibodies of different species and is not dependent on the antigenic specificity of the antibodies or on the nature of their heavy and light chains. Furthermore, it is shared with other non-immunoglobulin molecules such as chicken ovalbumin and β-galactosidase [23]. In the case of antibodies, the results suggest a primary role of tryptophan (Trp) residues buried within the molecule in the process of water oxidation, with particular emphasis on residues Trp36 and Trp37 that are conserved in more than 99% of antibodies [24]. In our opinion, the capacity of antibodies to oxidize water does not allow their identification as catalytic antibodies as far as this activity is not dependent on the nature of the genes that encode the variable regions of the antibodies and is not associated to any particular antigen specificity. This observation however documents the intrinsic protective role of antibodies under physiological conditions; a role that is independent of the capacity of antibodies to neutralize circulating exogenous antigens, to facilitate their endocytosis by antigen-presenting cells, and to participate in their elimination from the organism.

Production of H_2O_2 by anti-GPIIIa IgG (GPIIIa is a platelet antigen which is equivalent to to the β3 chain of integrins and associates with GPIIb to form a complex essential for platelet

activation) that arise in the course of HIV infection was implicated in the thrombocytopenic activity of anti-platelet antibodies [25].

Conclusion

Two types of catalytic antibodies can be distinguished. Firstly, 'induced' catalytic antibodies only appear upon active immunization using molecular mimics. The immunization with haptens that are analogs of transition states of chemical reactions, or with structures complementary to the active site of enzymes (variable regions of Ab1) would allow to fish out from a pool of available antibodies reactive antibodies prone to acquire a catalytic activity during the process of affinity maturation. In contrast, 'spontaneously' occurring catalytic antibodies are produced by the immune system in a constitutive manner under physiological conditions. The catalytic potential of such antibodies would then be inscribed in the repertoire of genes encoding the variable regions of the antibodies and might be inherited from primitive mechanisms of defense against pathogenic agents. The production of catalytic antibodies increases when the homeostasis of the immune system is perturbed, e.g. during pregnancy or during autoimmune disorders. The frequency of B-cell clones producing catalytic antibodies is also increased in NZB/W, MRL-lpr/lpr and SJL/J mice which are classical animal models for human autoimmune diseases [26]. The occurrence of lupus-like autoimmune manifestations in MRL-lpr/lpr mice is associated with a defect in the Fas-dependent apoptotic pathway and with the persistence in the periphery of deleterious autoreactive lymphocytes. Together, these observations suggest that B lymphocytes producing 'spontaneous' catalytic antibodies are subjected to a strong negative regulation under physiological conditions.

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