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Cloning of Human Anti-IgE Autoantibodies and Their Role in the Regulation of IgE Synthesis

Key Words

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Abstract

In vivo experiments using anti-IgE antibodies have clearly documented that they inhibit IgE production. In vitro experiments showed that not only IgE synthesis but also the effector phase of the allergic response may be influenced, because anti-IgE antibodies can prevent basophil sensitization with IgE or even remove IgE-receptor-bound IgE molecules. However, the question remains whether naturally occurring anti-IgE autoantibodies possess similar biological activity. To generate such antibodies for the necessary in vitro studies, we have cloned human Ig variable genes and selected anti-IgE antibodies using phage display libraries. Most of the human anti-IgE antibodies were anti-idiotypes, but anti-isotypes were also isolated.

Introduction

Our previous studies using murine anti-IgE antibodies have shown that they have variable and sometimes even opposite biological effects [1]. We found anti-IgE antibodies that were anaphylactogenic and some that were not. Interestingly, one antibody interfered with the binding of IgE to basophils, without recognizing the IgE receptor binding site. Because this antibody removes surface IgE it can be regarded as a 'desensitizing' antibody.

Similar multifunctional effects were observed for the binding of IgE to the low-affinity receptor CD23 [2, 3]. Some antibodies removed surface-associated IgE, others bound more to the cell surface of CD23+ cells by an as yet unknown, but eventually Fcγ-mediated effect. Other antibodies prevented IgE binding, and a fourth type did not influence the binding of IgE to CD23 at all.

Anti-IgE antibodies also varied greatly in their capacity to inhibit IgE synthesis [4]. A few antibodies were found

that even enhanced an interleukin-4 induced IgE synthesis in peripheral blood mononuclear cell (PBMC) cultures. Our murine anti-IgE monoclonal antibodies were all mapped for their domain specificity using recombinant IgE constant domains. From these data, it became clear that certain biological functions could be correlated to the domain specificity of the antibodies [3, 4]. Clearly some antibodies that recognized the third or second ε domain were not or were only weakly anaphylactogenic and also exhibited the most potent inhibition of IgE synthesis. Thus, it may be possible to select antibodies according to their epitope specificity to obtain optimal antibodies for in vivo treatment.

Among the murine monoclonal anti-IgE antibodies we tested, we have found none against the first IgE domain. In contrast, human serum containing naturally occurring anti-IgE autoantibodies, seems to contain antibodies that recognize all four ε domains. Interestingly, the ratios of antibodies against four ε domains vary in different patient groups, suggesting that differences in the amount of anti-IgE anti-

bodies and in their domain specificity may reflect different biological activity *in vivo* [5].

One of the major problems determining anti-IgE antibodies in serum is the fact that the antigen (IgE) as well as the autoantibody coexist in approximately equimolar concentrations and form immune complexes [6, 7]. Thus, antibodies binding to the relevant IgE epitopes may previously have not been detected diagnostically because they would be buried within immune complexes. For these reasons, it is also not possible to isolate anti-IgE autoantibodies and study their biological functions *in vitro* [7]. Thus, we decided to isolate human anti-IgE autoantibodies from the human genome using phage display libraries [8]. This technique enabled us to express antibody fragments from a cloned repertoire of an atopic individual [9]. Our results show that autoantibodies, especially anti-idiotypes as well as anti-IgE anti-isotype antibodies can be obtained by this technology.

Results and Discussion

For the construction of an Ig variable-gene combinatorial library, we selected a human donor with detectable serum anti-IgE autoantibodies. The donor was immunized with tetanus toxoid to ensure that the intended phage display library would contain an elevated proportion of specific antibodies against tetanus toxoid natural and nonspecific antibodies. A combinatorial library of V_H and V_L with V heavy-chain immunoglobulin genes was generated using the M13-based phage vector. For cloning the heavy-chain repertoire, we used a set of primers within the first constant domain of IgG1. This enhances the probability that the amplified V_H genes from B cells are those that have already encountered antigen. Using the phage system, Fab antibody fragments are expressed as a fusion protein with protein 3 of the M13 phage. There are only 3–4 copies/phage from this protein and due to a coinfection with a helper phage, one finds on average one Fab/phage, which forms a univalent recognition unit or a Phab. This means that Phabs selected via the antigen are at the same time selected for affinity. Phabs are then passed several times over the antigen bound to a solid-phase (called 'panning'), eluted, and again amplified, resulting in a library of antigen-specific phages.

The results obtained by our positive control, namely Phabs specific for tetanus toxin, revealed that by means of phage display libraries, very similar antibodies to those produced in humans can be obtained. We found a similar oligoclonality and the same affinity of Phabs, comparing them to antibodies in a commercial human antitetanus toxoid preparation. Interestingly, the toxin-specific phage library was

more efficient in neutralizing tetanus toxin than a combination of two neutralizing human monoclonal antitetanus toxin antibodies. These results are important for documenting that the antibodies isolated resemble specific antibodies in all important aspects [Lang et al., *in prep.*].

It is commonly realized nowadays that it is not practically possible to isolate antibodies against any given specificity from a nonimmune library. We were surprised by the great number of anti-IgE antibodies isolated from the library of the atopic donor. However, not every IgE myeloma was equally suitable to enrich the library for anti-IgE antibodies, because the majority of anti-IgE antibodies were actually anti-idiotypic antibodies, and only a few were ϵ isotype specific. Interestingly, in the library of this atopic individual, only antibodies against the second IgE domain were found. We are now screening phage display libraries from nonatopics to investigate whether these individuals encode antibodies against an epitope within the third ϵ domain, i.e. those exhibiting the most beneficial effects in our *in vitro* experiments.

These human recombinant anti-IgE antibodies will then be screened in different bioassays. For this purpose, the recombinant human Fab anti-IgE antibodies have to be transformed into bivalent or complete antibodies. This step is required, because univalent Fab fragments could not inhibit human IgE synthesis or trigger mediator release from human basophils. Our recent results using murine anti-IgE antibodies confirm this finding [4]. We demonstrated that Fab generated from monoclonal anti-IgE antibodies did not inhibit IgE synthesis, while the complete antibodies, or $F(ab')_2$ antibodies, were inhibitory [4]. The same principle holds true for the triggering of human basophils.

One of the most prominent questions is, of course, the mechanism of IgE synthesis inhibition. When we analyzed cells staining for both IgE and Bcl-2 by FACS analysis, it became clear that inhibitory antibodies down-regulated Bcl-2 expression. This suggests that anti-IgE antibodies down-regulate IgE synthesis by inducing apoptosis in the B cells. The few antibodies that enhanced human IgE synthesis did not show this effect on purified B cells, but only in unfractionated PBMC cell cultures. Thus, antibodies that are capable of cross-linking membrane IgE may induce apoptosis, while antibodies that cannot cross-link may nevertheless trigger surface-bound IgE on other effector cells, e.g. T cells, monocytes or basophils. In the case of T cells or basophils, such an interaction may lead to the production of cytokines, including interleukin-4, resulting in the indirect effect of IgE enhancement.

Our observation, that some anti-IgE antibodies can remove surface-bound IgE, even from human basophils, has

now also been reproduced at the molecular level. These desensitizing antibodies are also capable of removing IgE from recombinant α chains of the Fc ϵ RI bound on a solid phase. These data suggest that such anti-IgE antibodies may be used therapeutically, particularly in symptomatic patients.

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