
Naturally occurring blocking antibodies modulate immediate hypersensitivity responses in human filariasis.

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Abstracts

Although the basophils and mast cells of patients with chronic helminth infection are sensitized with specific IgE antibody and frequently exposed to parasite antigens in vivo, these patients rarely manifest allergic reactions to their parasites. To investigate the regulatory mechanisms limiting immediate hypersensitivity responsiveness in such patients, we used the in vitro antigen-in-phils as correlate of in vivo allergic response. For 13 elicited by graded doses of microfilarial antigen in the absence of serum or in the presence of normal human serum, autologous serum, or serum from other infected patients.

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Although the basophils and mast cells of patients with chronic helminth infection are sensitized with specific IgE antibody and frequently exposed to parasite antigens in vivo, these patients rarely manifest allergic reactions to their parasites. To investigate the regulatory mechanisms limiting immediate hypersensitivity reactivity in such patients, we used the in vitro antigen-induced histamine-release (HR) reaction of human basophils as a correlate of in vivo allergic responses. For 13 patients with bancroftian filariasis HR responses were elicited by graded doses of microfilarial antigen in the absence of serum or in the presence of normal human serum, autologous serum, or serum from other infected patients.

In all instances, sera from patients with filariasis contained a factor that specifically inhibited HR to parasite antigen. Normal sera had no such inhibitory effect, but sera from other filariasis patients inhibited as effectively as autologous serum. This HR blocking factor was heat stable (56°C × 2 hr) and nondialyzable. Its parasite antigen specificity was demonstrated by its inability to block the HR of patient cells triggered by anti-IgE antibody and its lack of inhibitory effect on the HR response of ragweed-sensitized cells reacted with ragweed antigen E. Fractionation of the sera by staphylococcus protein A-Sepharose chromatography showed that the blocking factor was an IgG antibody whose activity could be removed by specific immunoadsorption with filarial antigen. The levels of blocking antibody in the sera of these patients were high, comparable to those reported for atopic patients on immunotherapy regimens. These findings demonstrate that IgG blocking antibodies directed against parasite allergens are a regular component of the immune response to chronic filarial infection and suggest their potential role in vivo for specifically modulating allergic responsiveness to parasite antigens.

There are many common features between the host's immune response to parasitic helminths and the response of atopic individuals to extrinsic allergens. One of the most prominent of these is the production of high levels of specific IgE antibodies. In allergic individuals these antibodies, bound to the surface of mast cells and basophils, interact with allergens to initiate the release of histamine and other immediate hypersensitivity (IH) mediators whose effects define the acute allergic syndromes (allergic rhinitis, asthma, eczema, and urticaria). However, in patients with chronic helminth infections such allergic reactions are distinctly uncommon. Despite the facts that basophils and mast cells of these patients are present in normal numbers, are highly sensitized with specific parasite IgE (1), and in vivo are frequently exposed to parasite antigen (2, 3), clinically apparent allergic reactions to the parasite are rare (4, 5).

A number of different mechanisms have been proposed to account for this paradox. Recently, Mazinque et al. (4) have described a small m.w. product derived from 1 helminth species (Schistosoma mansoni) that was able to inhibit directly the degranulation of rat mast cells in vitro and in vivo. Such a mechanism could be of obvious importance in modulating reactivity to parasite antigens if these findings can be extended to other host-parasite systems. A 2nd mechanism that has been suggested involves competition for IgE receptor sites between mast cell and basophil membranes between the specific antigen-IgE antibodies and the "nonspecific" IgE produced when helminth infections induce polyclonal activation of IgE-secreting B cell clones (5). The potential of such a receptor saturation mechanism to inhibit specific antigen-induced histamine release (HR) has been shown in vitro with human basophils (7) but has yet to be successfully demonstrated in vivo (8).

The present study was designed to investigate a 3rd possibility—to account for this paradox; namely, that the sera from patients with chronic helminth infection (in this case, filariae) contain host-derived blocking factors capable of inhibiting the IH reaction between patients' sensitized basophils and parasite antigen. Our data demonstrate that such serum blocking factors are a regular feature of host response to filarial infection and that they can be characterized as IgG blocking antibodies. These antibodies develop naturally during chronic filarial infection and appear functionally similar to the blocking antibodies described previously in atopic individuals, especially those undergoing immunotherapy ("desensitization") treatment (9, 10).

MATERIALS AND METHODS

Patients. Thirteen adults (22 to 45 yr old) with chronic filariasis were studied. Their infections with Wuchereria bancrofti had been acquired.

Abbreviations used in this paper: ANP, antigen-neutralizing capacity; B.n., Brugia nematodes; CI, confidence interval; CFT, complement fixation test; DE, dermonecrotic; ELISA, enzyme-linked immunosorbent assay; HGG, human γ-globulin; HR, histamine release; IH, immediate hypersensitivity; IU, the amount of antigen required to elicit 20% HR; IgE, antigen E; ANCC, ANC of serum at time 0; 2014
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HR and its inhibition in patients with filariasis. Both antigens used in our system (filarial and ragweed), as well as the anti-IgE antibody, induced HR from appropriately sensitized cells in dose-dependent fashion (Fig. 1). Although peak HR to antigen did vary from patient to patient, cells of all patients with filariasis gave greater than 50% HR to some concentration of B.m.m.f. antigen, and cells of all patients with ragweed allergy showed at least 50% HR to AgE. The position of the dose-response curve on the abscissa reflects the relative "cell sensitivity" of the patients. Curves of individuals with more highly sensitized cells lie to the left of those less highly sensitized. (Neither B.m.m.f. antigen nor AgE induced "nonspecific" release of histamine from unsensitized cells (11); unpublished observations.)

The central finding of our study was the observation that in vitro the filarial antigen-induced HR of cells from patients with filariasis could be markedly inhibited by autologous serum. Such inhibition is illustrated in Figure 2, in which it can be seen that increasing concentrations of autologous serum progressively shift the antigen dose-response curve to the right. This shift increases the $A_20$ value (the concentration of antigen required for 20% HR), and for simplification, only such $A_20$ values will be used in subsequent figures to indicate the position of the dose-response curves.

For every patient studied (Fig. 3), the addition of autologous serum caused appreciable increases in the amount of filarial antigen required to elicit 20% HR. These increases in 50% autologous serum ranged between 1 and 4 logs (Fig. 3a), but in other experiments in which the reaction was carried out in 90% serum (an even better approximation of the in vivo state), complete abolition of responsiveness over the range of achievable antigen concentrations has generally been obtained. Normal serum had no such inhibitory effect on these same basophils (Fig. 3b); in fact, usually its presence caused somewhat enhanced HR responses, as has been described previously (9). In the 8 patients whose HR reactions to filarial antigen were studied in both autologous and normal human serum, the $A_20$ was high, ranging from 10 to 10,000 (Table I, top line).

Specificity of the HR inhibition by serum. Three sets of experiments were performed to evaluate the specificity of the HR blocking factors found in our patients' sera. In the first, the inhibition was shown to be not restricted to the autologous cell.
serum situation but readily transferable to the cells of other filaria-sensitized individuals. Figure 4a demonstrates this point with cells from 4 patients tested with a total of 6 different homologous immune sera from other filaria-infected persons. In general, the sera with the highest blocking activity in the autologous cell-serum systems were also the most effective in the homologous systems. Normal sera (this time derived from 5 different donors, not all with type AB blood) had no inhibitory effect on the response to filaria antigen in these same 4 sets of cells (Fig. 4b).

In the 2nd set of experiments, cells from ragweed-allergic individuals were reacted with ragweed AgE in the presence or absence of "inhibitory" sera from patients with filariasis. The results showed clearly that sera with high levels of blocking factor activity to IgE induced by filarial antigen had no effect on HR triggered by ragweed antigen. In fact, filariasis sera did not differ from either autologous or normal homologous sera in this ragweed-HR system (data not shown).

Finally, the serum blocking factor specificity was examined by using anti-IgE in parallel with filarial antigen as an alternate means for triggering HR from patient basophils. When the sera from 3 patients with filariasis were studied (Fig. 5), autologous serum (or its IgG fraction [see below and legend to Fig. 5]) regularly inhibited the HR induced by filarial antigen but had no such effect on HR triggered by anti-IgE antibody. The serum blocking factor, therefore, was filarial antigen specific.

Characterization of the serum blocking factor. Neither heating at 56°C for 2 hr nor extensive dialysis altered the specific blocking factor activity of sera from the filariasis patients (data not shown). For these reasons and because of its antigen specificity, we felt it likely that the serum blocking factor was IgG blocking antibody (16). Inhibitory sera were therefore fractionated over a protein A-Sepharose affinity column to yield IgG and IgG-depleted serum fractions. These fractions were

Figure 1. HR responses of basophils challenged in vitro with filarial antigen (panel A), ragweed antigen (panel B), or antibody to IgE (panel C). Cells in panel were taken from 3 Indian patients with filariasis; those in panel B were from 3 ragweed-sensitive North Americans; and those in panel C, from 2 filariasis patients and 1 normal North American.

Figure 2. Filarial antigen-induced HR responses of basophils from a patient with chronic filariasis. The presence of autologous serum inhibits the release of histamine, resulting in a shift of the dose-response curve to the right and an increase in the AUC value.
Figure 3. For all 13 patients studied, HR induced by filarial antigen was regularly exhibited by 50% autologous serum (see also increasing Ao values in Fig. 3a). No such inhibition was seen when cells of 8 of these 13 patients were tested in the presence of 50% normal human serum (Fig. 3b). The different Ao values in 0% serum indicate different levels of basophil sensitization with specific IgE by each of the patients.

serum fractions were titrated and tested as described above, it could be seen that absorption with specific filarial antigens removed nearly all blocking activity (Fig. 7). That this absorption was not the result of nonspecific binding to the matrix was indicated by the lack of absorption of blocking activity by the HGG immunosorbent.

DISCUSSION

Our data demonstrate unequivocally the presence of parasite antigen-specific IgG blocking antibodies in sera from patients with chronic filariasis and indicate for the first time that such antibodies are a regular component of the immune response to chronic helminth infection. The notion, however, that blocking antibody could be important in controlling allergic reactivity is certainly not new, the first description of such antibodies having been given by Noon (17) and Freeman (18) in 1911 from studies of grass pollen-immunized patients with allergic rhinitis. Indeed, the concept of blocking antibody still remains a guiding precept in the nonpharmacologic approach to controlling atopic disorders (19). The findings in our study, however, suggest that there are at least 2 important differences between the blocking antibody response of atopic individuals and that of patients with helminth infections. These differences, viz, the development of blocking antibodies as a "natural" concomitant of the host response to helminth infection and their abundance even in nonimmunized or untreated patients, imply that as a regulatory mechanism for immediate hypersensitivity reactions such antibodies may be much more biologically important for chronic helminth infections than they are for the allergic disorders.

Quantitation of blocking antibody has been a persistently difficult problem because the activity of this antibody is not defined physically (e.g., by immune precipitation, hemagglutination inhibition, etc.), but biologically by its effects on allergic responses. After in vitro basophil HR was established as a correlate of the in vivo allergic response, block antibody activity became more easily quantifiable by means.....the ANC of serum (20). Initially, this ANC was assessed by preincubating various concentrations of antigen for 30 or 60 min with either normal serum or autologous allergic serum before reacting this antigen-in-serum with sensitized cells. Later, however, May et al. (15) suggested that the ANC determined after preincubation of antigen with serum was really a measure of the "maximal binding capacity" of the serum and very likely overestimated the biologic importance of the blocking antibody detected, since no equivalent period of "preincubation" would exist in vivo. Their studies of allergic patients demonstrated that a serum's ANC determined in the absence of preincubation (ANC0) was appreciably less than that determined after a

Figure 4. Transfer of blocking activity. Filaria antigen-induced HR in cells of 4 patients with filariasis was inhibited by each of 6 different homologous sera from other patients with filariasis (panel a); 5 homologous normal sera had no such effect (panel b).
Figure 5. Blocking activity was restricted to HR induced by filarial antigen when cells from 3 patients with filariasis were triggered either by filarial antigen (solid symbols) or by anti-IgE antibody (open symbols). Since IgE in serum will react with the anti-IgE antibody, it can interfere with the antibody’s triggering of basophils. When total serum IgE was low (as in patient A with 300 IU/mI), this potential interference presented no problem and the specificity of the blocking factor could be demonstrated in whole autologous serum. With high levels of total serum IgE, however, (1400 IU/mI for patient B and 2400 IU/1 for patient C) the IgE blocking antibody (see text) had to be separated from serum IgE by protein A-Sepharose chromatography before the filarial antigen specificity of the serum blocking factor could be demonstrated (panels B and C).

Figure 6. Two sera with HR blocking activity were fractionated over staph A-Sepharose columns and the fractions (IgG-depleted (open symbols) and IgG-rich (solid symbols)) tested for blocking factor activity.

Figure 7. Blocking activity was removed by absorption of the IgG fraction of inhibitory sera with a filarial antigen immunosorbent (solid symbols) but not with the “irrelevant” HGG immunosorbent (open symbols).

preincubation step (range of 1 to 75 times less in the 17 patients studied).

Table 1 utilizes ANC values to compare directly the amount of blocking activity in the sera of patients with filarial infection with the amount of serum blocking activity in patients with allergic disorders. Although high titers of blocking antibody have been shown unequivocally to develop in allergic patients treated on immunotherapy regimens (9, 10, 21), it is clear from Table 1 (which summarizes studies from the literature with “pretreatment” ANC values) that before treatment there is little “natural” or spontaneous development of such antibodies in these individuals. In contrast to the low ANC values in untreated allergic individuals, patients with filariasis showed compa-

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... large amounts of serum blocking antibody, which had developed spontaneously during infection. Whether the absence of appreciable blocking antibody in atopic individuals is simply a reflection of antigen dose, antigen quality, route of administration, or persistence, still the natural development of blocking antibody in patients with helminth infection appears to be a major difference between the responses of these patients and those with allergic disorders.

Blocking antibodies constitute an effective control mechanism for modulating IH reactions in vitro. Their effectiveness in vivo, however, has been subject to much debate (19, 22), although they do develop in atopic patients on immunotherapy, their presence cannot always be related to the symptomatic improvement experienced by these patients. On the other hand, given the abundance of such antibodies in patients with filariasis, it is our hypothesis that these IgG antibodies serve in vivo to limit allergic responses to parasite antigens and thereby account in large measure for the clinical paradox that allergic reactions to these antigens are rare. Although a number of mechanisms for modulating IH responsiveness in helminth infections have been proposed, including the parasite-derived mast cell-releasing factor (4) and competitive receptor binding (7) described above, a regulatory mechanism involving IgG blocking antibody would have the distinct theoretical advantage that it is parasite antigen specific. Such antigen-specific modulating mechanisms have already been described for delayed hypersensitivity immune responses in both filariasis (23, 24) and other helminth infections of man (25-28), and quite likely it is the antigen specificity of these immunoregulatory mechanisms that underlies the unique biologic relationship between host and parasite.

This role for IgG antibodies would, of course, be in addition to others already described as likely to be functioning during such infections; e.g., those involved in protective immunity (29, 30), those activating complement to produce anaphylotoxins and other inflammatory mediators (31), and those participating in antibody-dependent cellular cytotoxicity against the parasite (32, 33). Interestingly, in preliminary studies we have found that the titers of blocking antibody in individual sera do not correlate with IgG antibody titers to the same crude antigen preparation assessed by other techniques (enzyme-linked immunosorbent assay and radioimmunoassay). We are therefore currently investigating the possibilities that the blocking antibody may be restricted to a particular subclass of IgG, or that they may be of any IgG subclass but are directed only against the allergens in the complex antigen preparations.

The value to the host of specific and effective regulatory mechanisms for IH responsiveness is obvious. While IH reactivity doubtlessly play important, although still largely undefined, roles in the protective immune response to helminth parasites (34-36), it is clear that uncontrolled anaphylactic reactivity to parasite products would be detrimental. In our in vitro studies, the inhibitory effect of blocking antibody on HR could generally be overcome by increasing concentrations of antigen; in vivo similar gradients of antigen must exist, with the greatest concentration being in the immediate vicinity of the parasite. Therefore, the potential in vivo role of blocking antibodies need not necessarily be to abolish IH reactivity to parasite antigen, but rather to contain or limit its extent. At the site of the parasite (e.g., where it penetrates the skin or where it lies in the lymphatic) antigen concentration might be great enough to induce a local IH inflammatory response, but as the concentration of antigen progressively diminishes with distance from the parasite, blocking antibody might reduce the level of “available” antigen below that needed to trigger mediator release. Thus, the extent of the IH reaction would have been effectively limited to that region that was biologically useful for the host.

Finally, an understanding of the modulation of IH responsiveness in parasite infections may also be bear significantly on prospects for improving the immunologic control of atopic diseases. Although the immunotherapy regimens currently used are of acknowledged value in controlling allergic reactions to certain allergens, even this control is often only partial; indeed, it is generally nowhere near so complete as that exerted by the host in avoiding allergic reactivity to parasite allergens during chronic helminth infection. If, as our findings suggest, these latter control mechanisms depend on blocking antibody, then definition of the characteristics of this antibody and the factors responsible for its induction will be particularly important. Such analysis should increase our understanding of the immunoregulatory mechanisms utilized by the host to accommodate to chronic parasitic infection and at the same time give particular insight into the pathogenetic and control aspects of IH responses both in relation to the helminth parasites and in relation to the common nonparasite allergens.

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REFERENCES


15. May, C. D., M. S. Lymann, R. Alberts, and N. Aduna. 1972. On the measurement...
OTTESEN, KUMARASWAMI, PARANJAPE, POINDEXTER, AND TRIPATHY


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