

Saturation of Immunoglobulin E (IgE) Binding Sites by Polyclonal IgE Does Not Explain the Protective Effect of Helminth Infections against Atopy

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One hypothesis for the decreased rates of atopy observed among helminth-infected individuals is that parasite-induced polyclonal immunoglobulin E (IgE) outcompetes allergen-specific IgE for FcεRI binding on basophils and mast cells. In experiments with fresh blood drawn from filaria-infected patients, we found no association between ratios of polyclonal to *Brugia malayi* antigen (BmAg)-specific IgE (range, 14:1 to 388:1) and basophil responses to BmAg as measured by histamine release. Using serum samples from a filaria-infected patient who also had dust mite (*Dermatophagoides pteronyssinus*)-specific IgE antibodies from time points with various ratios of polyclonal to *D. pteronyssinus*-specific IgE (16:1 to 86:1), we demonstrated that increased ratios of polyclonal to *D. pteronyssinus*-specific IgE did not attenuate basophil sensitization as measured by *D. pteronyssinus*-specific histamine release. Suppression of histamine release was likely not observed in either of these sets of experiments because polyclonal to antigen-specific IgE ratios were not sufficiently high, as concurrent passive sensitization of basophil experiments required ratios of polyclonal to antigen-specific IgE of greater than 500:1 to suppress basophil histamine release. Further, the intensity of IgE staining in basophil populations from 20 patients with active filaria infections correlated strongly with total serum IgE levels ($\rho = 0.698$; $P = 0.0024$) with no plateau in intensity of IgE staining, even though some patients had total IgE levels of greater than 10,000 ng/ml. Our data therefore suggest that in helminth infections (and in filarial infections in particular), the ratios of polyclonal to allergen-specific IgE rarely reach those levels necessary to inhibit allergen-specific IgE-FcεRI binding and to suppress allergen-induced degranulation of mast cells and basophils.

Helminth infections have been associated with decreased rates of asthma (19, 27) and allergy (4, 13, 14, 22, 30). Among the explanations proposed to explain these findings, the most longstanding is the IgE-blocking hypothesis, which posits that high polyclonal IgE levels induced by helminth infections suppress allergic responses by saturating the high-affinity IgE FcεRI on basophils and mast cells (5, 11, 14). By preventing sufficient numbers of allergen-specific IgE molecules from binding to these cells, it has been argued that high polyclonal IgE levels inhibit allergen-induced basophil and mast cell degranulation.

One of the first studies suggestive of IgE blocking demonstrated that allergen-specific skin test reactivity after intradermal injection of serum containing allergen-specific IgE (the Prausnitz-Küstner test) was inhibited when sera containing high concentrations of non-allergen-specific IgE was first injected into the skin (28). This finding was supported by reports that intradermal sensitization cannot be induced in rats (21) or humans (23) with IgE myeloma and that in vitro sensitization of mast cells from chopped lung fragments is blocked when the tissue is initially incubated with serum from West African subjects with high total IgE levels (6). On the basis of these and other reports, in 1976 the possibility of deliberately infecting

allergic patients with parasites for the purpose of inducing high polyclonal IgE antibody levels was suggested (11).

Epidemiologic and experimental data obtained since then, however, have provided mixed support for this hypothesis. Of epidemiologic studies that take into account confounding variables, two studies have found a correlation between IgE levels and decreased allergy and asthma (4, 22), whereas two other studies have not (27, 30). Further, while treatment of intestinal geohelminths in Venezuelan children resulted in decreased polyclonal IgE levels, increased allergen skin test reactivity, and increased positivity of Prausnitz-Küstner testing (13), high levels of polyclonal IgE induced by helminth infection did not prevent active sensitization when allergen-specific IgE antibody is produced in vivo in rats (10). Also, unlike IgE myeloma patients, cells from Waorani Indians with high IgE levels can be passively sensitized with sera containing allergen-specific IgE (12).

In this study, we sought to determine the validity of the IgE-blocking hypothesis. First we evaluated whether a relationship exists between the ratio of polyclonal IgE to antigen-specific IgE and antigen-driven histamine release in patients with filarial infections that typically induce high polyclonal IgE levels. If large quantities of polyclonal IgE can saturate FcεRI, then an inverse relationship should exist between polyclonal to antigen-specific IgE ratios and histamine release in response to specific antigen. Second, we sensitized basophils from normal donors with various mixtures of purified human myeloma IgE and filaria patient sera to determine the in vitro ratio of poly-

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clonal to specific IgE necessary to inhibit antigen-specific histamine release. Finally, we studied whether there is a relationship between serum levels of IgE and quantities of IgE bound to basophils. While it is well established that FcεRI expression on basophils is upregulated in proportion to serum IgE levels in allergic and normal control patients (16, 18), such a relationship has not been substantially confirmed in helminth infections. Evidence that quantities of IgE bound to basophils increase with increasing serum IgE levels in helminth-infected patients suggests that saturation of FcεRI is not easily achieved *in vivo*.

MATERIALS AND METHODS

Subjects. Filaria-infected and uninfected individuals were recruited from the Clinical Parasitology Unit (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, MD) and from the Department of Transfusion Medicine (NIH), respectively, under protocols approved by the NIAID Institutional Review Board. The diagnosis of filaria infection was based on (i) positive identification of appropriate parasite or parasite DNA in blood, skin snips, or tissue biopsy by microscopy or PCR, (ii) positive circulating antigen test for *Wuchereria bancrofti*, (iii) positive antifilarial serology plus Calabar swellings and response to therapy for *Loa loa*, or (iv) positive antifilarial serology plus either Mazzotti reaction or ophthalmologic exam consistent with onchocercal eye disease for *Onchocerca volvulus* (7).

Basophil sensitization. The protocol for sensitizing basophils was modified from those of Pruzansky et al. (24) and Schroeder and Kagey-Sobotka (26). Heparinized venous blood was collected from normal human donors, and 50×10^6 to 100×10^6 peripheral blood mononuclear cells (PBMC) were separated using a Ficoll diatrizoate gradient at 4°C (LSM; ICN Biomedicals, Aurora, OH). PBMC were washed in PAG buffer (PIPES buffer [Sigma-Aldrich, St. Louis, MO] containing 0.1% human serum albumin [Calbiochem, San Diego, CA] and 0.1% D-glucose [Mallinckrodt, Paris, KY]) and then centrifuged at $900 \times g$ for 10 min. Cells were resuspended in phosphate-buffered saline (PBS; BioWhittaker, Walkersville, MD) and then centrifuged again at $900 \times g$ for 10 min. To strip IgE molecules from their high-affinity receptors on basophils, cells were next resuspended in 3 ml of lactic acid buffer comprising 4.1 mM lactic acid (Calbiochem), 140 mM NaCl (Mallinckrodt), and 5 mM KCl (Mallinckrodt) and adjusted to pH 3.9. After 60 s of incubation at room temperature, 12 ml of PAG buffer was added and the cells were centrifuged at $900 \times g$ for 10 min. Cells were washed twice in PAG buffer and then resuspended in sensitization buffer (PAG buffer with 4 mM EDTA [Sigma-Aldrich]) and 10 U/ml of heparin (American Pharmaceutical Partners, Schaumburg, IL) at 13.3×10^6 cells/ml. Patient plasma was then added to the cells in sensitization buffer at a volume ratio of 1:4. For the polyclonal IgE saturation experiments, purified human myeloma IgE (Chemicon International, Temecula, CA) was added in various concentrations together with patient plasma. Cells were then incubated at 37°C for 1 h, washed twice with PAG buffer, and resuspended in histamine release buffer (Beckman Coulter, Marseille, France) at 5×10^6 cells/ml.

Histamine release. Histamine release reactions were conducted either on sensitized basophils or on 200-μl aliquots of heparinized whole blood diluted 1:7 with histamine release buffer. Studies of whole blood were done within 2 h of blood drawing from the patient. Cells were incubated for 30 min at 37°C with histamine release buffer alone and various concentrations of either soluble *Brugia malayi* antigen (BmAg; prepared as described previously [20] and chosen because of extensive cross-reactivity among filarial species) or soluble *Dermatophagoides pteronyssinus* antigen (AlkAbello, Round Rock, TX). In experiments using sensitized basophils, cells were also incubated with rat IgG1 anti-human IgE (Biosource, Camarillo, CA) or nonspecific rat IgG1 (Biosource) at 2 μg/ml as positive and negative controls to ensure sensitization had taken place. One aliquot of cells was diluted 1:20 in distilled water and then frozen and thawed twice to give a measure of total histamine stores. Histamine concentrations were then measured using a commercially available histamine enzyme immunoassay kit (Beckman Coulter). Values obtained under different conditions were divided by total histamine and multiplied by 100 to obtain percentages of total histamine released. The highest level of histamine detected from a dose-response curve of increasing antigen concentration was used to determine the maximum percentage of total histamine released. The threshold concentration of antigen required to induce histamine release was defined as the lowest concentration of antigen that induced >10% total histamine release.

Quantification of BmAg-specific IgE, *D. pteronyssinus*-specific IgE, and total (polyclonal) IgE. Measurements of BmAg-specific IgE and *D. pteronyssinus*-specific IgE were performed by enzyme-linked immunosorbent assay (ELISA). Prior to measuring BmAg-specific IgE, IgG was adsorbed by incubating serum samples with GammaBind G Sepharose (Pharmacia Biotech, Uppsala, Sweden) overnight at 4°C. Flat-bottom plates (Immulon 4; Dynatech, Chantilly, VA) were coated overnight at 4°C with either *D. pteronyssinus* or BmAg at 10 μg/ml in PBS (BioWhittaker, Walkersville, MD), followed by washing with PBS and 0.05% Tween (Sigma Chemical). Plates were then blocked with PBS–5% bovine serum albumin (BSA)–0.05% Tween for 2 h at 37°C and washed again. Serum samples diluted 1:10 in ELISA diluent (PBS, 1% BSA, 0.05% Tween 20), and standards were then added and the mixture was incubated overnight at 4°C. Plates were then washed and incubated with biotinylated goat anti-human IgE (Fortron Bioscience, Morrisville, NC) at 3 μg/ml in ELISA diluent for 2 h at 37°C for detection. Following washing, 1 μg/ml of alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) in ELISA diluent was added and the mixture was incubated for 1 h at 37°C. Plates were again washed, and *p*-nitrophenylphosphate disodium (Sigma Chemical) was added at 1 mg/ml in sodium carbonate buffer (KD Medical, Columbia, MD). Colorimetric development was detected at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA), and absolute values were assessed based on interpolation from standard curves.

Total serum IgE levels were measured by the Laboratory of Clinical Pathology (Clinical Center, NIH) using an Immulite 2000 Immunoassay System (Diagnostic Products Corporation, Los Angeles, CA).

Cell preparation and fixation for flow cytometry. Heparinized venous blood was collected and PBMC separated using LSM at 4°C. Cells were washed and then cultured at 2×10^6 /ml in Iscove's Dulbecco modified Eagle medium (Biofluids, Rockville, MD) supplemented with 10% heat-inactivated fetal calf serum (HyClone, Logan, UT), 1% L-glutamine (Biofluids), 1% insulin-transferrin-selenium medium (Biofluids), and 80 μg/ml gentamicin (BioWhittaker) for 2 h at 37°C. Cells were then transferred to 15-ml conical tubes, washed twice in PBS, and then fixed in PBS containing 4% paraformaldehyde (Sigma Chemical) for 5 min. Cells were then washed in PBS containing 0.1% BSA (Sigma Chemical), resuspended in PBS–10% dimethyl sulfoxide (Fischer Scientific, Fair Lawn, NJ), and cryopreserved at –70°C.

Flow cytometry. Fixed cells were thawed, washed with PBS–0.1% BSA, and then incubated in PBS containing 0.1% saponin (CalBiochem)–1% BSA at 4°C for 1 h. Cells were then stained with fluorescein isothiocyanate-conjugated goat anti-human IgE (Biosource), Cy-chrome-conjugated mouse IgG1 anti-human CD2 (BD PharMingen, San Diego, CA), tricolor-conjugated mouse IgG2a anti-human CD14 (Caltag, Burlingame, CA), tricolor-conjugated mouse IgG1 anti-human CD16 (Caltag), and Cy-chrome-conjugated mouse IgG1κ anti-human CD19 (BD PharMingen). After staining, cells were washed twice with PBS–0.1% saponin, resuspended in PBS, and analyzed using a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA) and CellQuest software (Becton Dickinson). Prior to running patient samples, all antibodies were individually titrated with positive and negative control cells for optimal sensitivity and specificity.

Statistical analysis. Comparisons between groups of unpaired data were performed using the nonparametric Mann-Whitney U test, and correlations were derived using the Spearman rank test. All statistics were performed using StatView5 (SAS Institute, Cary, NC).

RESULTS

High polyclonal to specific IgE ratios do not correlate with decreased histamine release in filaria-infected patients. To determine whether high ratios of polyclonal IgE to antigen-specific IgE correlate with decreased basophil responsiveness, whole blood samples from 13 filaria-infected individuals with measurable BmAg-specific IgE (Table 1) were incubated for 30 min alone or with serial 10-fold dilutions of BmAg (from 100 to 1×10^{-7} μg/ml) and assayed for released histamine. While these patients had very high polyclonal IgE levels (most with levels of >1,000 ng/ml and three with levels of >10,000 ng/ml) and a wide range of polyclonal to BmAg-specific IgE ratios (from 14:1 to 388:1), there was no relationship between these ratios and the ability of basophils from these patients to release histamine when stimulated with BmAg in terms of

TABLE 1. Characteristics of filaria-infected patients for whom histamine release assays were conducted

Patient	Diagnosis	Level (ng/ml) of:		Polyclonal IgE to BmAg IgE ratio	BmAg threshold for histamine release (ng/ml)	BmAg level (ng/ml) at which maximum % of histamine is released	% of Maximum histamine released
		Polyclonal IgE	BmAg IgE				
1	<i>L. loa</i>	18,612	48	388:1	0.1	100	57
2	<i>O. volvulus</i>	12,512	36	348:1	0.001	10	63
3	<i>O. volvulus</i>	4,812	17	283:1	1	100	49
4	<i>L. loa</i>	1,240	9	138:1	100	100	12
5	<i>L. loa</i>	1,459	12	122:1	0.1	100	57
6	<i>L. loa</i>	27,723	254	109:1	0.1	10	60
7	<i>L. loa</i>	6,634	73	91:1	0.01	1	56
8	<i>L. loa</i>	1,908	27	71:1	0.001	0.1	64
9	<i>W. bancrofti</i>	3,453	51	68:1	0.001	0.1	67
10	<i>O. volvulus</i>	351	7	50:1	0.01	10	61
11	<i>L. loa</i>	146	5	29:1	0.1	10	35
12	<i>L. loa</i>	368	22	17:1	0.1	100	63
13	<i>L. loa</i>	110	8	14:1	NA ^a	0.1	4

^a NA, not applicable.

maximum percent histamine release ($\rho = -0.003$; $P = 0.99$; Fig. 1). Additionally, there was no correlation between polyclonal to BmAg-specific IgE ratios and the threshold concentration of BmAg required to induce >10% total histamine release ($\rho = 0.26$, $P = 0.38$, Table 1). All three patients with polyclonal to BmAg-specific IgE ratios of greater than 200:1 released substantial amounts of histamine in response to BmAg.

Sensitization of normal basophils with plasma containing increasing ratios of polyclonal IgE to *D. pteronyssinus*-specific IgE does not result in a decrease in *D. pteronyssinus*-triggered histamine release. We conducted basophil sensitization experiments to determine whether changes in polyclonal to allergen-specific IgE ratios alter basophil responsiveness to specific allergen. After screening stored serum samples from filaria-infected patients, serially collected serum samples from one patient coinfecting with *L. loa* and *O. volvulus* were found to have high levels of *D. pteronyssinus*-specific IgE and large variations in polyclonal IgE levels (Fig. 2) and were used to sensitize basophils from an uninfected nonallergic human donor with no detectable *D. pteronyssinus*-specific IgE. Sensitized basophils were then stimulated with anti-human IgE, control antibody, or *D. pteronyssinus* antigen at 0.001, 0.1, and 10 $\mu\text{g/ml}$.

Histamine release curves were similar for all serum samples used in the sensitization step despite their differences in polyclonal to *D. pteronyssinus*-specific IgE ratios (Fig. 2), with no

significant differences in maximal histamine release ($P > 0.6$) or shifts in the curves between cells sensitized with high ratios of polyclonal IgE to *D. pteronyssinus*-specific IgE sera (ratios, >50:1) and those sensitized with low ratios of polyclonal IgE to *D. pteronyssinus*-specific IgE sera (ratios, <50:1). Anti-IgE stimulation caused 7% total histamine release from cells sensitized with buffer alone and 44% to 54% total histamine release from cells sensitized with the serum samples, confirming that sensitization with serum samples had been successful. As expected, cells from the normal donor that were not stripped of their IgE molecules, as well as those that were stripped and sensitized only with buffer, did not release histamine in response to *D. pteronyssinus* antigen (data not shown).

Basophil sensitization to a specific antigen can be blocked, but only when basophils are sensitized with extremely high ratios of polyclonal to specific IgE. Basophils from three normal uninfected donors were sensitized with plasma from a *Loa*-infected patient (patient 6, Table 1) mixed with increasing concentrations of myeloma IgE. After sensitization, cells were stimulated with 10 $\mu\text{g/ml}$ of BmAg for 30 min and then assayed for released histamine. Quantities of released histamine could

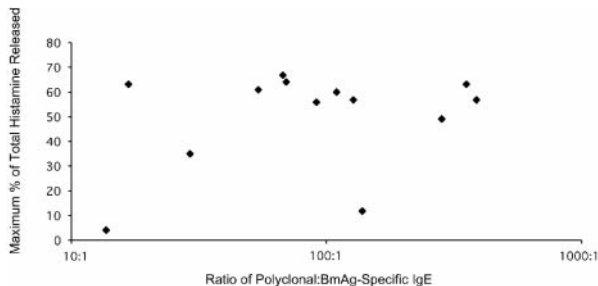


FIG. 1. Histamine release in whole blood from filaria-infected patients after incubation with BmAg as a function of the ratios of polyclonal to BmAg-specific IgE.

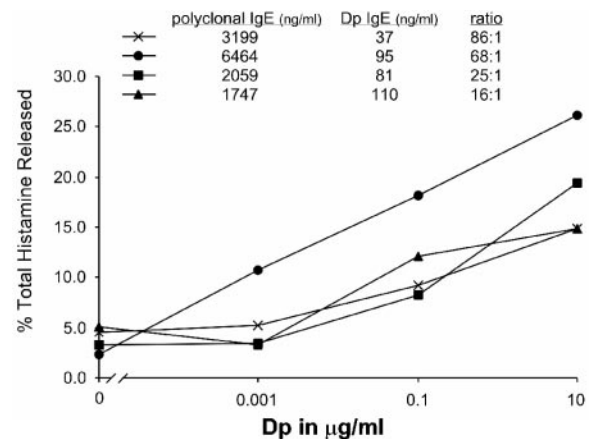


FIG. 2. *D. pteronyssinus* antigen-triggered histamine release from basophils sensitized with serum samples of a filaria-infected patient whose polyclonal to *D. pteronyssinus* (Dp)-specific IgE ratios varied over time.

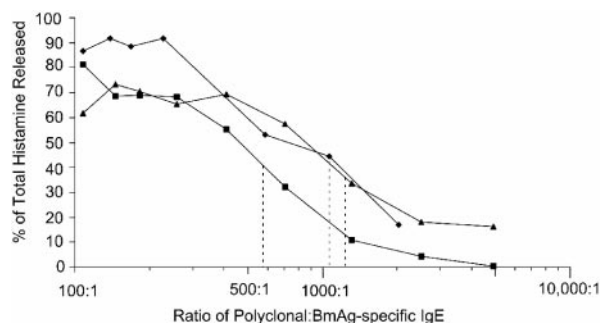


FIG. 3. BmAg-triggered histamine release from normal basophils after sensitization with filaria patient serum mixed with increasing amounts of polyclonal myeloma IgE. The experiment was done three times, with each line representing a different normal donor's basophils. The far left point on all three curves denotes BmAg-triggered histamine release after sensitization with the filaria patient serum alone (patient 6 in Table 1, whose serum had a polyclonal to BmAg-specific ratio of 109:1). Dashed lines indicate the polyclonal to BmAg-specific ratios at which BmAg-triggered histamine release was half-maximal.

be decreased to less than half of maximal histamine release, but only when cells were sensitized with polyclonal to specific IgE ratios of greater than 500:1. Moreover, histamine release could be completely abrogated when cells were sensitized with ratios of polyclonal to specific IgE in excess of 1,000:1 (Fig. 3). All cells sensitized with patient plasma had significantly greater histamine release than those that were simply stripped of IgE and sensitized with only buffer (range, 71% to 96% of total histamine release for samples sensitized with plasma versus 42% to 52% for samples sensitized with only buffer), confirming that sensitization was successful.

Quantities of IgE bound to basophils correlate with serum IgE levels. To determine whether the amount of IgE bound to each basophil correlates with serum IgE levels, basophils from PBMC isolated from 20 filaria-infected patients (patients 1 to 13 in Table 1 plus 7 others) were identified by flow cytometry analysis and measured for mean fluorescence intensity after staining with fluorescently labeled anti-IgE. While there was no relationship between serum levels of polyclonal IgE and the percentages of basophils in the peripheral circulation ($\rho = -0.051$; $P = 0.82$; data not shown), there was a strong correlation between total serum IgE levels and intensity of IgE staining on basophils ($\rho = 0.698$; $P = 0.002$; Fig. 4). Additionally, although some patients had total IgE levels of greater than 10,000 ng/ml, there was no evidence of a plateau in the intensity of IgE staining, suggesting that saturation of FcεRI does not occur in vivo.

DISCUSSION

Prevalence of asthma and allergic diseases is greater in industrialized countries and urban areas than in developing countries and rural areas (1, 9). Epidemiologic studies have demonstrated associations between exposure to infectious agents and related products, including endotoxin, tuberculosis, hepatitis A, *Toxoplasma gondii*, and *Helicobacter pylori* and decreased rates of asthma and allergy. In addition to bacterial and viral infections, helminth infections have been shown in a multitude of studies to be associated with decreased rates of

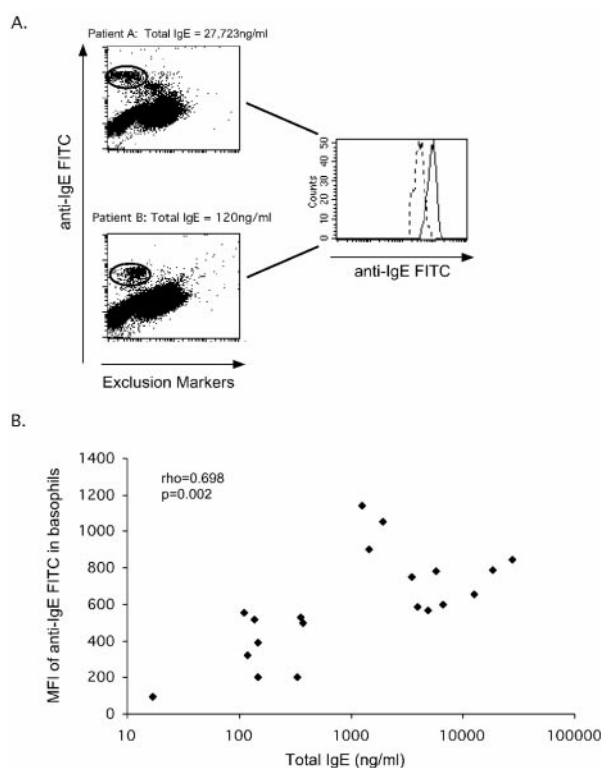


FIG. 4. (A) Representative dot plots of basophil identification by flow cytometry in one patient with high total serum IgE (patient A) and another with low total serum IgE (patient B). Basophils were identified by gating on PBMC that stained positively for surface IgE and negatively for CD2, CD14, CD16, and CD19 (left plots; encircled areas represent basophil populations), and the surface expression of IgE on basophils was analyzed by measuring the intensity of anti-IgE staining of the gated basophil populations (right histogram; solid line denotes patient A, dashed line denotes patient B). (B) Basophil cell surface expression of IgE measured as the mean fluorescence intensity (MFI) of anti-IgE staining of basophils as a function of total serum IgE levels in 20 filaria-infected patients. FITC, fluorescein isothiocyanate.

asthma and allergy (4, 13, 14, 19, 22, 27, 30; reviewed in reference 29). Immunologic mechanisms proposed to explain the protective effect of helminth infections on the risk of asthma and allergy include the production of low-affinity allergen-cross-reacting IgE antibody, production of IgG4-blocking antibody, upregulation of interleukin-10, and/or an anti-inflammatory network, and the IgE-blocking hypothesis (reviewed in reference 32). Suggested over a quarter century ago (5, 11), the IgE-blocking hypothesis postulates that high polyclonal IgE levels induced by helminth infections suppress allergic responses by saturating the high-affinity IgE receptors on basophils and mast cells, thereby preventing sufficient numbers of allergen-specific IgE molecules from binding to these effector cells. In this study, we have demonstrated that while saturation of IgE binding sites by high ratios of polyclonal to specific IgE is physically possible in vitro, this phenomenon likely occurs only rarely in helminth-infected patients.

First, we showed that there was no association between ratios of polyclonal IgE to specific IgE and basophil responses to specific antigen as measured by histamine release. Although the filaria-infected patients we studied had extremely high

polyclonal IgE levels (most with levels of >1,000 ng/ml and three with levels of >10,000 ng/ml) and wide ranges in the ratios of polyclonal to BmAg-specific IgE (14:1 to 388:1), there was no relationship between these ratios and histamine release after stimulation with parasite antigen. As these studies were conducted within 2 h of blood drawing, the basophil surface expression of IgE molecules was likely the same as the basophil surface phenotype in vivo. The lack of an association between polyclonal to BmAg-specific IgE ratios and BmAg-triggered histamine release demonstrates that high levels of polyclonal IgE in vivo did not saturate basophil FcεRI in patients and did not prevent BmAg-specific IgE binding on basophils or alter the ability of BmAg-specific IgE molecules to be cross-linked by antigen. By sensitizing basophils from a normal, nonallergic donor with plasma samples taken from a filaria-infected patient who had various polyclonal to *D. pteronyssinus*-specific IgE ratios over time, we also demonstrated that high polyclonal to antigen-specific IgE ratios do not inhibit antigen-specific IgE-mediated histamine release even when the antigen used is an allergen.

Suppression of histamine release was likely not observed in the BmAg and *D. pteronyssinus* antigen histamine release experiments because the polyclonal to antigen-specific IgE ratios in the patients studied were not sufficiently high for physiologic FcεRI saturation to occur. By sensitizing normal donor basophils with increasing concentrations of polyclonal to antigen-specific IgE, we were able to suppress antigen-specific histamine responses in vitro when cells were sensitized with polyclonal to specific IgE ratios of greater than 500:1 and were able to completely extinguish these responses with polyclonal to antigen-specific IgE ratios of greater than 1,000:1. Although extremely high, these values are consistent with what is known about basic basophil biology. While FcεRI densities can range up to 1×10^6 per cell (15, 18), with most individuals having several hundred thousand IgE receptors per basophil (3), the number of IgE cross-links necessary to trigger basophil activation is only about 1,000 molecules per cell (15, 17). Thus, in an average person, one can predict that polyclonal to specific IgE ratios of several hundred thousand to a thousand (i.e., several hundred to one) would be necessary to inhibit specific IgE responses by saturation of FcεRI, which is consistent with our experimental findings.

In our saturation experiments, cells were incubated with high concentrations of IgE molecules for only 1 h. Because a human study conducted over 3 months showed that FcεRI expression on basophils varies in proportion to serum IgE levels (16), it is likely that the 500:1 polyclonal to antigen-specific IgE ratio obtained from our saturation experiments is actually an underestimation of the actual polyclonal to antigen-specific IgE ratio necessary in vivo to saturate IgE receptors and prevent antigen-specific IgE binding to the point that antigen-driven histamine release is suppressed. While it is fairly well established that IgE levels modulate FcεRI expression in basophils, with the exception of one study that included six helminth-infected patients (25), prior work showing the linear correlation between levels of serum IgE and basophil-bound IgE has been done primarily with allergic populations (16, 18). In this study, we found a strong correlation between serum levels of total IgE and the surface expression of IgE on basophils in filaria-infected patients, suggesting that, as in al-

lergic patients, serum IgE levels drive FcεRI expression on basophils in helminth-infected patients. As there was no apparent plateau of IgE binding at high concentrations of serum IgE, it appears unlikely that IgE receptors were saturated even at high serum IgE concentrations. Although our experiments were conducted with basophils, our results are likely generalizable to mast cells as well, because mast cells have similar numbers of FcεRI per cell (2) and upregulate surface FcεRI density in response to increases in serum IgE levels (8, 31).

In conclusion, we have shown that high polyclonal to specific IgE ratios do not correlate with decreased histamine release in response to either filarial antigen or dust mite antigen in filaria-infected patients. We demonstrated that suppression of histamine release was not observed in these experiments because polyclonal to antigen-specific IgE ratios were not high enough, as concurrent passive sensitization of basophil experiments required ratios of at least 500:1 to suppress antigen-specific basophil histamine release. Further, as we have provided evidence that serum IgE levels modulate FcεRI expression on basophils in helminth-infected patients, it is probable that polyclonal to specific-IgE ratios of even greater than 500:1 are necessary to inhibit antigen-specific histamine release from basophils in helminth-infected patients. While the percentage of helminth-infected patients that fit these criteria is not known, it is probably low. Less than 10% of 173 helminth-infected patients evaluated at the NIH Clinical Parasitology Unit over the past 2 decades had polyclonal IgE levels of >10,000 ng/ml (unpublished data). Since most dust mite-allergic patients will have *Dermatophagoides*-specific IgE levels of at least 10 ng/ml (approximate level of class II *D. pteronyssinus*-specific IgE RAST category), only a few percent of patients with helminth infections and dust mite-specific IgE would be expected to have ratios of polyclonal to *D. pteronyssinus*-specific IgE of >1,000:1. Thus, saturation of IgE binding sites by polyclonal IgE in helminth-infected patients, while possible, likely occurs only rarely and is not likely the primary mechanism whereby most helminth-infected patients are protected from allergy and asthma.

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