

Identification of Recombinant Filarial Proteins Capable of Inducing Polyclonal and Antigen-Specific IgE and IgG4 Antibodies

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Filarial infection is characterized by an immune response associated with the production of Ag-specific IgG4 and IgE and IL-4 and IL-5. To identify filarial Ags capable of inducing such responses and to analyze the role Ags themselves play in sustaining it, 24 recombinant filarial parasite proteins were screened for their ability to be recognized by sera from 67 individuals with tissue-invasive filarial infections. Among the recombinant proteins that were recognized by IgG4 or IgE Abs in 25% of the sera or more, two were selected on the basis of their ability to elicit polyclonal and Ag-specific IgE/IgG4 Abs in vitro. Ov27 (analogous to Ov7/cystatin, a cysteine protease inhibitor) and OvD5B (analogous to Ov33, an aspartyl protease inhibitor) induced both a polyclonal and Ag-specific IgE/IgG4 response that was blocked by neutralizing Abs to IL-4 and to IL-13 or by soluble IL-4 receptors. Recombinant human IFN- γ and IL-12 also led to a decrease in the production of polyclonal and Ag-specific IgE/IgG4 Abs. In addition, these two recombinant proteins preferentially stimulated the secretion of IL-4, IL-5, and IL-10 (in contrast to IFN- γ). The data suggest that certain epitopes on filarial Ags preferentially elicit a Th2-type response and provide an in vitro model for dissecting the mechanisms underlying this preferential response. *The Journal of Immunology*, 1995, 155: 1316–1325.

Although the number of tissue-invasive helminths pathogenic for humans is large and the biology of each is diverse, infections with helminths are characteristically accompanied by high levels of IgE and IgG4 Ab production, tissue and peripheral blood eosinophilia, and the participation of inflammatory mediator-rich basophils and mast cells. In parasitic helminth infection, these types of responses can induce pathologic reactions similar to those seen in atopic disorders (1, 2), although clinical manifestations of acute allergy are not common in patients with chronic infections with filariae, the reason being attributed to a control of allergic reactivity by blocking Abs of the IgG4 subclass (3). Typically, individuals with tissue-invasive helminth infection have eosinophil and IgE/IgG4 levels several times greater than uninfected

individuals (3). The factors responsible for this degree of elevation are not well defined, although they most likely reflect a clonal expansion of Th2-type CD4⁺ T cells capable of producing IL-4 and IL-5, cytokines implicated in the induction of IgE/IgG4 and eosinophilia (4–7). Th2-type CD4⁺ T cells can also secrete IL-13, a cytokine that acts on monocytes and B cells in a manner similar to IL-4 (8).

This study attempts to examine the role that parasite Ags themselves play in determining the specific B cell and T cell responses in helminth infections. First, a large panel of recombinant parasite Ags was screened to identify those recognized by IgE and IgG4 Abs of infected patients. The recombinant filarial proteins were then utilized in an in vitro system to examine the induction of both IgG4 and IgE Abs as well as the cytokines responsible for these Ab responses.

Materials and Methods

Sera and peripheral blood mononuclear cell preparation

Sera were obtained from individuals with *Loa loa* ($n = 40$) or *Onchocerca volvulus* ($n = 27$) and from normal nonexposed individuals ($n = 10$). A majority of patients (91%) were expatriates who acquired their infections in West Africa; the clinical details of these patients have

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been described previously (9–11). Sera from these individuals were used to screen a panel of recombinant proteins (see below). Cryopreserved PBMCs from the same patients and from normal individuals, previously isolated on Ficoll-diatrizoate gradient, were thawed, washed, and cultured as described below.

Recombinant parasite proteins

The purified recombinant parasite proteins used in this study were obtained as fusion proteins or as fusion products from which the nonparasite protein had been cleaved. They were obtained from diverse species of filariae pathogenic to humans, including *O. volvulus*, *L. loa*, and *Brugia malayi*, or potentially pathogenic to humans, (e.g., *Dirofilaria immitis*). OvSOD (GenBank X57105; K. Henkle-Dürksen, Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany), paramyosin (GenBank M29733; L. McReynolds, New England Biolabs, Beverly, MA), Di-5 (GenBank M95173; L. McReynolds), L1-Di5 (L. McReynolds), “43kDa” (GenBank L18869; D. Freedman, University of Alabama, Birmingham, AL), and OG#11 (GenBank K01050; J. Catmull, University of Iowa, Iowa City, IA) were purified, fusion-cleaved proteins. BpL4 (EMBL X68190; R. Maizel, Imperial College of Science, Technology and Medicine, London, United Kingdom) was fused to 6-histidine. SPX-1 (GenBank M98813; W. Piessens, Harvard School of Public Health, Boston, MA), C2.2 (C. Carlow, New England Biolabs), Ov14, Ov2, and B20 (A. E. Bianco, Liverpool School of Tropical Medicine, Liverpool, United Kingdom), OvT1-M3 and OvT1-M4 (GenBank U12681; A. Scott, Johns Hopkins University, Baltimore, MD), OI3 (F. Perler, New England Biolabs), OvD5B (F. Perler), chitinase (GenBank M73689; F. Perler), RAL-2 (GenBank A60758; T. Unnasch, University of Alabama), RAL-6 (GenBank U12440; T. Unnasch), Ov27 (GenBank M37105; J. Bradley, Imperial College of Science, Technology and Medicine), were maltose binding protein (MBP)²-fused proteins. Ov9 (GenBank U01099; S. Lustigman, New York Blood Center, New York, NY), Ov103 (GenBank M55155; S. Lustigman), E15, E18, and E21 (A. E. Bianco), OvL3-1 (GenBank V14530; R. Lucius, Universität Hohenheim, Stuttgart, Germany), were glutathione-S-transferase (GST)-fused proteins. Controls were ELISA diluent for fusion-cleaved proteins and 6-HIS-fused proteins: MBP for MBP-fused proteins, and GST for GST-fused proteins. Each was at concentrations similar to the fusion-proteins (see below).

In vitro antibody production

Cryopreserved PBMCs were thawed, washed, and adjusted to a concentration of 2×10^6 cells/ml. The cells were cultured in 48-well plates (Costar, Cambridge, MA) at a final volume of 0.5 ml of Iscove's Dulbecco modified medium (Biofluids, Rockville, MD) supplemented with 1% L-glutamine (Biofluids), 80 μ g/ml gentamicin (BioWhittaker, Walkersville, MD), 1% insulin-transferrin-selenium medium (Biofluids), and 10% FCS (HyClone, Logan, UT). PBMCs were cultured with recombinant parasite proteins (at optimal concentrations) or with the relevant fusion vector proteins (MBP or GST) as controls. In some experiments, cultures were performed in the presence of recombinant human (rh) IFN- γ (Genzyme, Cambridge, MA) and rhIL-12 (a gift of S. Wolfe, Genetics Institute, Cambridge, MA) or neutralizing Ab to hIL-4 (Genzyme), hIL-13 (Peprotech, Rocky Hill, NJ), hIFN- γ (Interferon Sciences, New Brunswick, NJ), or hIL-12 (a gift of S. Wolfe). Recombinant human IL-4 soluble receptors (shIL-4R) were purchased from R&D Systems (Minneapolis, MN). Control rabbit, rat, or mouse antisera (to irrelevant Ag specificities) were used where appropriate. Cultures were conducted at 37°C with 5% CO₂. Supernatants were collected at day 14 and frozen until assayed.

In vitro cytokine production and cytokine assays

PBMC (2.5×10^6 /ml, 750 μ l/well) were cultured in complete RPMI 1640 medium (BioWhittaker) containing 80 μ g/ml gentamicin (BioWhittaker), 25 mM HEPES (Biofluids), 1% L-glutamine (Biofluids), and 10%

endotoxin-free FCS (Life Technologies, Grand Island, NY) and placed in 24-well culture plates (Costar).

Cytokine levels were measured by ELISA and calculated from interpolation from standard curves based on recombinant cytokine dilutions as described elsewhere (12). PBMC culture supernatants were assayed as follows: IL-4, day 1; IL-10, day 2; IL-5 and IFN- γ , day 5.

ELISA for polyclonal and Ag-specific IgG subclasses and IgE

Polyclonal IgG4 and IgE were measured by ELISA using techniques essentially as previously described (13), with the modification that the coating anti-human IgE was E25 (kindly provided by P. Jardieu, Genentech, San Francisco, CA). Briefly, Immulon-4 plates (Dynatech, Springfield, VA) were coated with mouse mAbs to either human IgG4 Fc (HP6023) (Hybridoma Reagent Labs, Baltimore, MD) at 1 μ g/ml or to human IgE (E25) at 3 μ g/ml. Serum analyses were performed using 50 μ l of samples to be assayed for IgG4 (serum dilution = 1/25) or IgE (IgG-depleted sera diluted 1/5). Culture supernatants were assayed undiluted.

Ag-specific IgG4 and IgE Abs were detected by ELISA using 0.5 μ g/ml recombinant parasite protein or control fusion-protein in NaHCO₃/Na₂CO₃, pH 9.6, buffer (4°C). The plates were washed, and undiluted culture supernatants were incubated for 2 h at 37°C and overnight at 4°C. For the detection of specific IgG subclass Abs, mouse anti-human IgG Abs were added as follows: mouse HP6069 IgG1-Fc (1 μ g/ml), HP6014 IgG2-Fd (0.5 μ g/ml), HP6047 IgG3-hinge (0.5 μ g/ml), or HP6023 IgG4-Fc (0.5 μ g/ml) (2 h at 37°C). After washing, 0.5 μ g/ml alkaline-phosphatase (AP) goat anti-mouse IgG (Jackson, West Grove, PA) was also added for 2 h at 37°C. The AP substrate was *p*-nitrophenyl phosphate disodium 1 mg/ml (Sigma Chemical Co., St. Louis, MO). Data are expressed as OD_{405–650} ratios (recombinant protein-OD values:fusion molecule-OD values). OD_{405–650} ratios were calculated (from duplicates) for each serum/protein analysis vs fusion-protein molecule values when recombinant proteins were fused (MBP or GST) or vs uninfected normal donor serum values when recombinant proteins were cleaved. For the detection of specific IgE Abs, biotinylated (14) purified goat anti-human IgE Ab (G82320015; BiosPacific, Emeryville, CA) at 1 μ g/ml was applied for 2 h (37°C), followed by streptavidin-AP (1/500) (Life Technologies) for 1 h at room temperature. Color development and analysis were done as described above.

Statistical analysis

Data were expressed as means $\pm$ SEM unless otherwise stated. Statistical analysis was performed using Student's *t*-test for normally distributed values and by the Mann-Whitney *U* test when the data were not distributed normally.

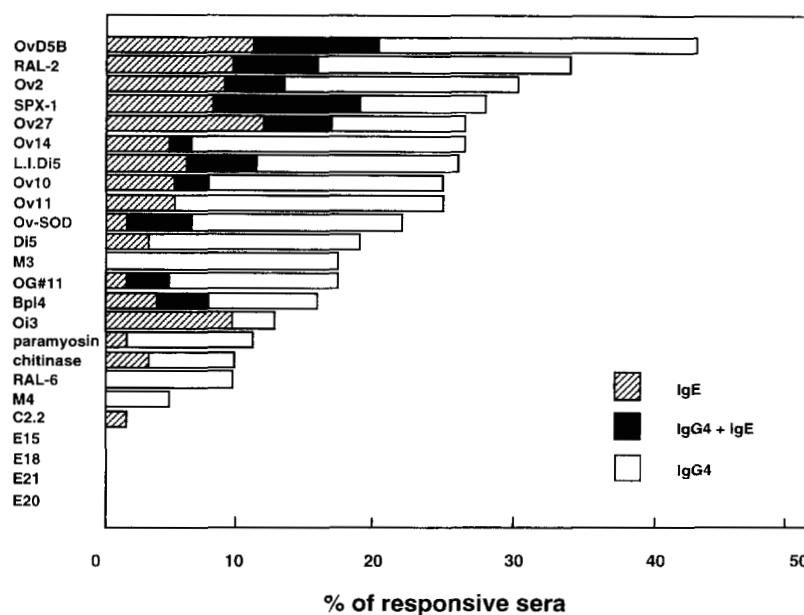
Results

Identification of recombinant filarial proteins capable of being recognized by IgG4 and/or IgE Abs in the serum of *L. loa*- or *O. volvulus*-infected patients

To delineate those Ags capable of being recognized by specific IgE and/or IgG4 Abs, 24 recombinant filarial proteins were screened with sera obtained from untreated *L. loa* ($n = 40$)- or *O. volvulus* ($n = 27$)-infected individuals using Ag-specific ELISAs. The 24 recombinant proteins were variably recognized by protein-specific IgG4 and/or IgE Abs in the sera of filaria-infected individuals (Fig. 1): four were recognized by neither IgE nor IgG4 Abs from all sera tested, whereas 20 were recognized by two or more of the sera. The six most commonly recognized recombinant proteins were OvD5B, RAL-2, Ov2, SPX-1, Ov27, and Ov14. One recombinant protein (M3) induced responses largely restricted to individuals with *O. volvulus* infections. This Ag was recognized by 10/27 (37%) *O. volvulus*

² Abbreviations used in this paper: MBP, maltose binding protein; AP, alkaline phosphatase; GST, glutathione-S-transferase; h, human, as in hIFN- γ ; sIL-4R, soluble IL-4 receptors.

FIGURE 1. IgE and/or IgG4 Ab responses to recombinant filarial proteins. A panel of 67 sera from filaria-infected patients was screened for specific IgE and IgG4 Abs using a bank of 24 recombinant filarial proteins. Specific IgG4 and/or IgE Abs were detected by means of ELISA, and results are expressed as OD ratios (see *Materials and Methods*). Histograms represent the percentage of sera containing only IgG4 (□), only IgE (▨), or both IgG4 and IgE (■) Abs specific to each of the listed recombinant proteins.



patients and by only one *L. loa* patient; these responses were restricted to the IgG4 isotype. Also restricted to the IgG4 isotype were the RAL-6 and M4 proteins. Of the 67 sera tested, 14 (20.9%) did not contain IgE or IgG4 Abs to any of the 24 recombinant proteins (data not shown).

Four additional recombinant parasite proteins were screened for Ab reactivity to a panel of five sera that had reacted with at least five of the previously described recombinant parasite proteins; two of the four additional molecules (Ov9 and Ov103) were also recognized by either IgE or IgG4 serum Abs, whereas the two others ("43 kDa" and OvL3.1) were not (data not shown).

Recombinant filarial proteins that induce both in vivo and in vitro Ag-specific IgG4 and IgE Abs

Four recombinant proteins (OvD5B, RAL-2, SPX-1, and Ov27) were selected to be assayed in vitro for induction of specific and polyclonal IgE and IgG4 Abs. Each of these four recombinant proteins (all MBP-fused) was recognized by IgE and/or IgG4 Abs in $\geq 25\%$ of the 67 assayed sera. Unfractionated PBMCs from *L. loa*- or *O. volvulus*-infected individuals ($n = 19$) whose sera contained specific IgG4 and/or IgE Abs to an array of the selected recombinant proteins were thawed, washed, and cultured with various concentrations of proteins or with MBP as a control. PBMCs from five uninfected donors were also stimulated under the same conditions.

Two of the four recombinant proteins (OvD5B and Ov27) were capable of inducing IgE and/or IgG Abs in vitro in a consistent and reproducible pattern, ranging from 22 to 74% of the individuals tested (data not shown). Ov27 induced in vitro IgG4/IgE Ab production in 14/19 (73.7%) individuals and OvD5B in 11/19 (57.9%) individuals. Ab

production was not seen in PBMCs from uninfected normal individuals with either Ov27 or OvD5B (data not shown). MBP itself elicited neither IgE nor IgG4 Ab (data not shown). Figure 2 shows the dose-dependent relationship between the recombinant protein (OvD5B or Ov27) concentrations and the production of Ab-specific (Fig. 2, A and B) and of polyclonal (Fig. 2, C and D) IgE/IgG4. As seen, both recombinant proteins induced specific IgG4 (Fig. 2A) and IgE (Fig. 2B) in vitro in a dose-dependent fashion. OvD5B preferentially stimulated the production of polyclonal IgG4 (Fig. 2C), whereas Ov27 preferentially induced polyclonal IgE (Fig. 2D). Optimal Ab production was obtained using protein concentrations of 0.001 $\mu\text{g/ml}$ for Ov27 and 0.01 $\mu\text{g/ml}$ for OvD5B; these doses were subsequently chosen as working concentrations. To examine the ability of these rAgs to induce isotypes other than IgE and IgG4, the polyclonal IgG subclass levels were assessed in response to OvD5B (not shown) and Ov27 (Fig. 3). As seen, in 15/19 individuals showing a polyclonal IgG response to Ov27, when there was significant IgG4 response, there were little or no IgG1, -2, or -3 Abs. In contrast, in those individuals in whom IgG4 was poorly induced, IgG1 and IgG2 were primarily secreted. In those individuals in whom IgG4 was not detected, all three other IgG isotypes were secreted.

Ov27 and OvD5B rAgs induce IgG4 and IgE Ab production in vitro in an IL-4- and/or IL-13-dependent manner

To investigate the role of IL-4 in promoting IgE and IgG4 Abs in response to either Ov27 or OvD5B, these recombinant proteins were used to stimulate unfractionated PBMCs from filarial-infected patients at defined optimal

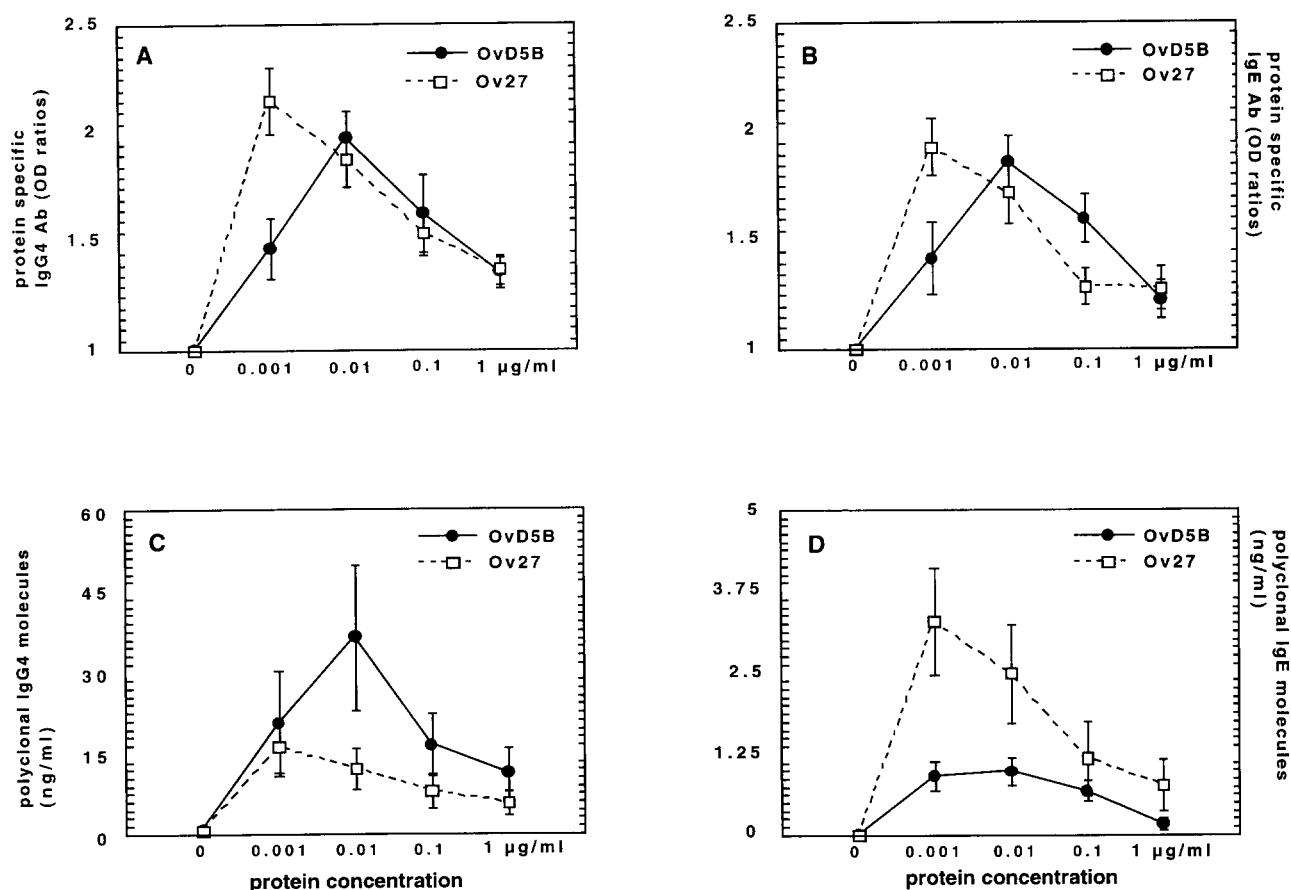


FIGURE 2. Ov27 and OvD5B induce in vitro production of IgE and/or IgG4 Ab in a dose-dependent fashion. Thawed, unfractionated PBMCs from filaria-infected patients (whose sera contained Ov27- and/or OvD5B-specific IgE and/or IgG4 Abs) were assayed in vitro in the presence of increasing concentrations of MBP-Ov27, MBP-OvD5B, or control MBP molecule (0–1 µg/ml; x-axis). Data are expressed as OD ratios for Ag-specific IgG4 (A) and IgE (B) and in ng/ml for polyclonal IgG4 (C) and IgE (D). Results are plotted as mean $\pm$ SEM of 14 individuals for Ov27 and of 11 individuals for OvD5B.

concentrations in the presence or absence of neutralizing anti-IL-4 Abs. Ov27 and OvD5B exhibited stimulatory activity for IgG4 and IgE production in a similar pattern (Fig. 2). The addition of neutralizing anti-IL-4 Abs was able to partially, although significantly ($p \leq 0.001$), decrease the secretion of protein-specific IgG4 Abs (Fig. 4A), protein-specific IgE Abs (Fig. 4B), polyclonal IgG4 Abs (Fig. 4C), and polyclonal IgE Abs (Fig. 4D) in 19 individual experiments. These results were confirmed and extended by adding recombinant human soluble IL-4 receptor (sIL-4R) to Ov27- and OvD5B-stimulated patient PBMCs (Fig. 5). A significant ($p \leq 0.05$) neutralization of both protein-specific IgG4 Abs (Fig. 5, *top panel*) and IgE (Fig. 5, *bottom panel*) resulted from the addition of sIL-4R in six individual sets of experiments; the mean percentage of inhibition for IgE was 41% (ranging from 20–53%); for IgG4, it was 35% (ranging from 13–62%).

To investigate the role of IL-13 in promoting IgE and IgG4 Abs in response to either Ov27 or OvD5B, rAg-stimulated patient PBMCs were cocultured with neutralizing anti-IL-13 Abs. The addition of neutralizing anti-

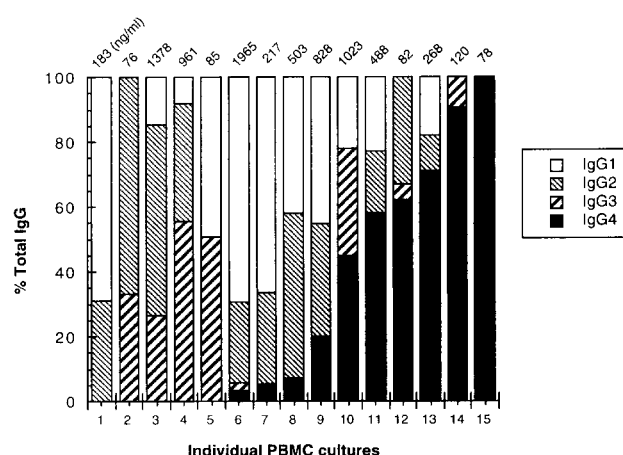
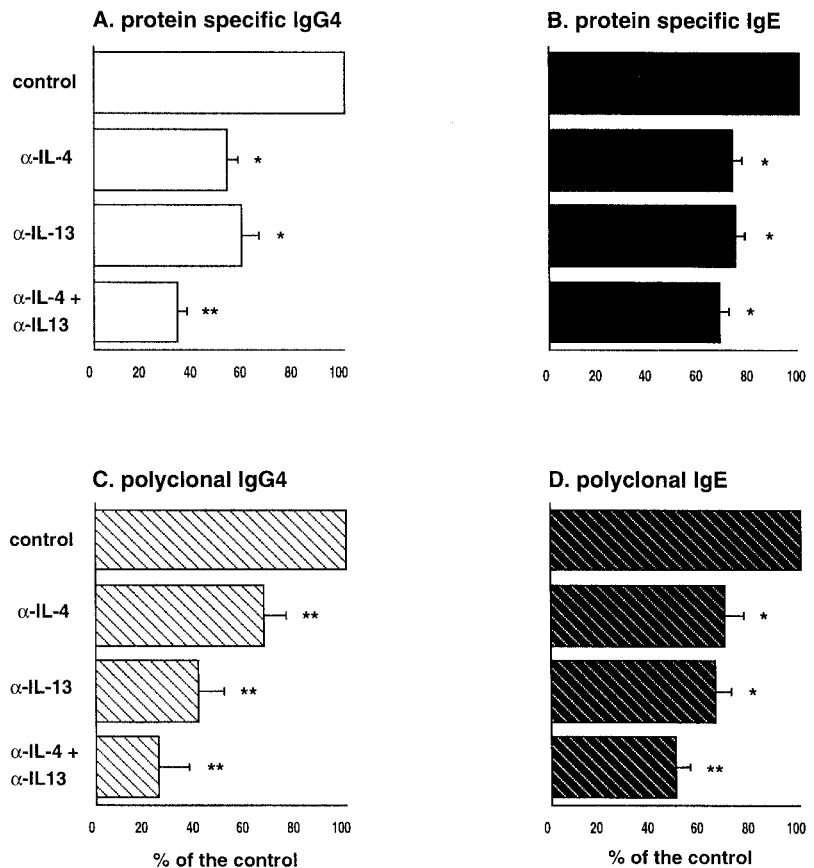


FIGURE 3. Polyclonal IgG subclasses induced by Ov27. PBMCs from filarial-infected patients were stimulated for 14 days with optimal concentrations of Ov27. The amounts of IgG1 (□), IgG2 (▨), IgG3 (▩), and IgG4 (■) for 15 individuals' PBMCs are expressed as a percent of the total IgG induced. At the *top* of each histogram is the actual level of IgG secreted (ng/ml) in each individual culture.

FIGURE 4. Ov27 and OvD5B induce IgG4 and/or IgE Ab secretion in vitro in IL-4- and/or IL-13-dependent fashions. Thawed, unfractionated PBMCs from filaria-infected patients were stimulated for 14 days with optimal concentrations of Ov27 or OvD5B in the presence of polyclonal rabbit anti-human IL-4 and/or anti-IL-13 Abs or control rabbit Abs. Results from the 10 included experiments are expressed as mean $\pm$ SEM percentage of the control (Ag + control Ab). The degree of significance is as follows: *, significantly different from control only; **, significantly different from the other data in the same panel.



IL-13 Abs resulted in a significant decrease ($p \leq 0.001$) of specific anti-Ov27 and anti-OVD5B IgG4 and IgE Ab production in the 19 individual PBMCs tested. This decrease is comparable to the one induced by anti-IL-4 Abs ($p \leq 0.001$) (Fig. 4, B–D) except for rAg-specific IgG4 Abs, where anti-IL-13 Abs neutralized Ov27- and OvD5B-specific IgG4 Abs to a significantly greater extent than anti-IL-4 Abs ($p \leq 0.001$) (Fig. 4A). Figure 4 also shows that, although all decreases are equally significant from the control responses, anti-IL-4 or anti-IL-13 Abs neutralized IgG4 (rAg-specific and polyclonal) Abs (Fig. 4, A and C) to a greater degree than IgE Abs (Fig. 4, B and D). Anti-IL-4 and anti-IL-13 Abs synergized to neutralize rAg-specific and polyclonal IgG4 Abs ($p \leq 0.001$) (Fig. 4, A and B) along with rAg-specific IgE Abs ($p \leq 0.01$) (Fig. 4C).

Ov27- and OvD5B-induced IgG4 and IgE Ab production is also affected by IFN- γ or IL-12 and by Abs to these cytokines

To investigate the role of IFN- γ and IL-12 in the regulation of the rAg-dependent IgG4 and IgE Ab production in vitro, PBMCs from filaria-infected patients were stimu-

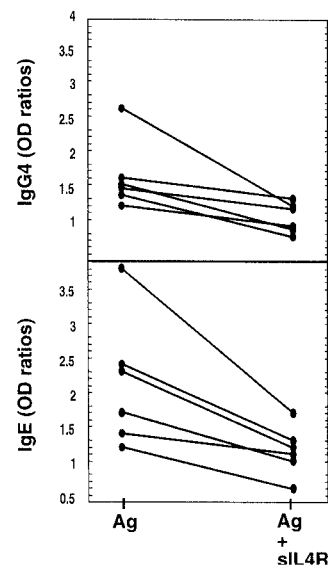


FIGURE 5. Recombinant human soluble IL-4 receptor molecules can partly inhibit Ov27- and OvD5B-induced IgG4 (top panel) and IgE (bottom panel) Ab in vitro production. Thawed, unfractionated PBMCs from six filaria-infected patients were stimulated for 14 days with optimal concentrations of Ov27 (0.001 μ g/ml) or OvD5B (0.01 μ g/ml) with or without 5 ng/ml sIL-4R.

Table 1. The effect of rhIFN- γ or rhIL-12 on Ov27- and OvD5B-induced IgG4 and IgE antibody secretion^a

Ab Specificity	Patient No.	Culture Conditions OD Ratios ^b (% of Inhibition)		
		rAg Only	rAg + rhIFN- γ	rAg + rhIL-12
IgG4	1	1.64	1.1 (32.9%)	1.02 (37.8%)
	2	2	0.95 (52.5%)	1.15 (31.3%)
	3	1.6	1 (37.5%)	2.02 (21.4%)
IgE	1	2.8	1.1 (60.7%)	2.02 (21.4%)
	2	2.7	1 (63.0%)	ND ^c
	3	3.15	0.76 (75.9%)	ND
	4	2.7	ND	1.4 (48.1%)
	5	2.2	ND	1.22 (44.6%)

^a Patient PBMCs (2×10^6 /ml, 500 μ l) were cultured for 14 days in the presence of either Ov27 (0.001 μ g/ml) or OvD5B (0.01 μ g/ml) and rhIFN- γ (50 IU/ml) or rhIL-12 (50 IU/ml).

^b Results are expressed as OD ratios (see *Materials and Methods*).

^c Not done.

lated with Ov27 and OvD5B in the presence or absence of rhIFN- γ or IL-12 or of neutralizing Abs to these cytokines.

The addition of rhIFN- γ (50 IU/ml) or of rhIL-12 (50 IU/ml) led to a decrease of Ov27- and OvD5B-induced polyclonal and rAg-specific IgG4/IgE production whenever the rAg-induced Ab production was detectable (OD ratio ≥ 1.5). As shown in Table 1, IFN- γ reduced rAg-specific IgG4 levels (by 33–52%); this inhibitory effect appears to be even more dramatic for rAg-specific IgE values (61–76% reduction). Although to a lesser extent than IFN- γ , IL-12 inhibited rAg-specific IgG4 and IgE Abs (31–42% and 21–48% reduction, respectively). Interestingly, although the addition of anti-IFN- γ Ab to Ov27- or OvD5B-stimulated cells induced a moderate (but not significant) increase of polyclonal IgG4 and IgE Abs, it exerted little or no effect on rAg-specific IgG4 and IgE Abs. Neutralizing anti-IL-12 Ab had little effect on polyclonal and rAg-specific IgG4/IgE production in the seven individuals studied (data not shown).

Ov27 and OvD5B induce filaria-infected patient PBMCs to secrete cytokines

To examine the presence of T cell epitopes in Ov27 and OvD5B Ags, PBMCs from nine filaria-infected patients were stimulated with various concentrations of the recombinant proteins, and the supernatants were analyzed for IL-4, IL-5, IL-10, and IFN- γ . IL-4 was detected in all 6 Ov27-stimulated cell cultures and in 3/5 OvD5B-stimulated cell cultures; IL-5 in 5/9 and 7/9 supernatants, respectively; IL-10 in 6/9 and 7/9 supernatants; and IFN- γ in 5/9 culture supernatants (in both types) (data not shown). At least two of these four cytokines were detected in every assayed culture supernatant. The relationship between each of the cytokines to the production of other cytokines was next examined (Fig. 6). As seen, there was a clear polarization of these cytokine responses: if IL-4, IL-5, or IL-10 was produced in response to a given Ag, IFN- γ was generally not induced. Conversely, in those PBMCs in

which the Ags induced IFN- γ , little or no IL-5 and/or IL-4 or IL-10 was produced. Of note, however, was the observation that not all of the high IL-4 producers also produced high levels of IL-5 (Fig. 6, inset).

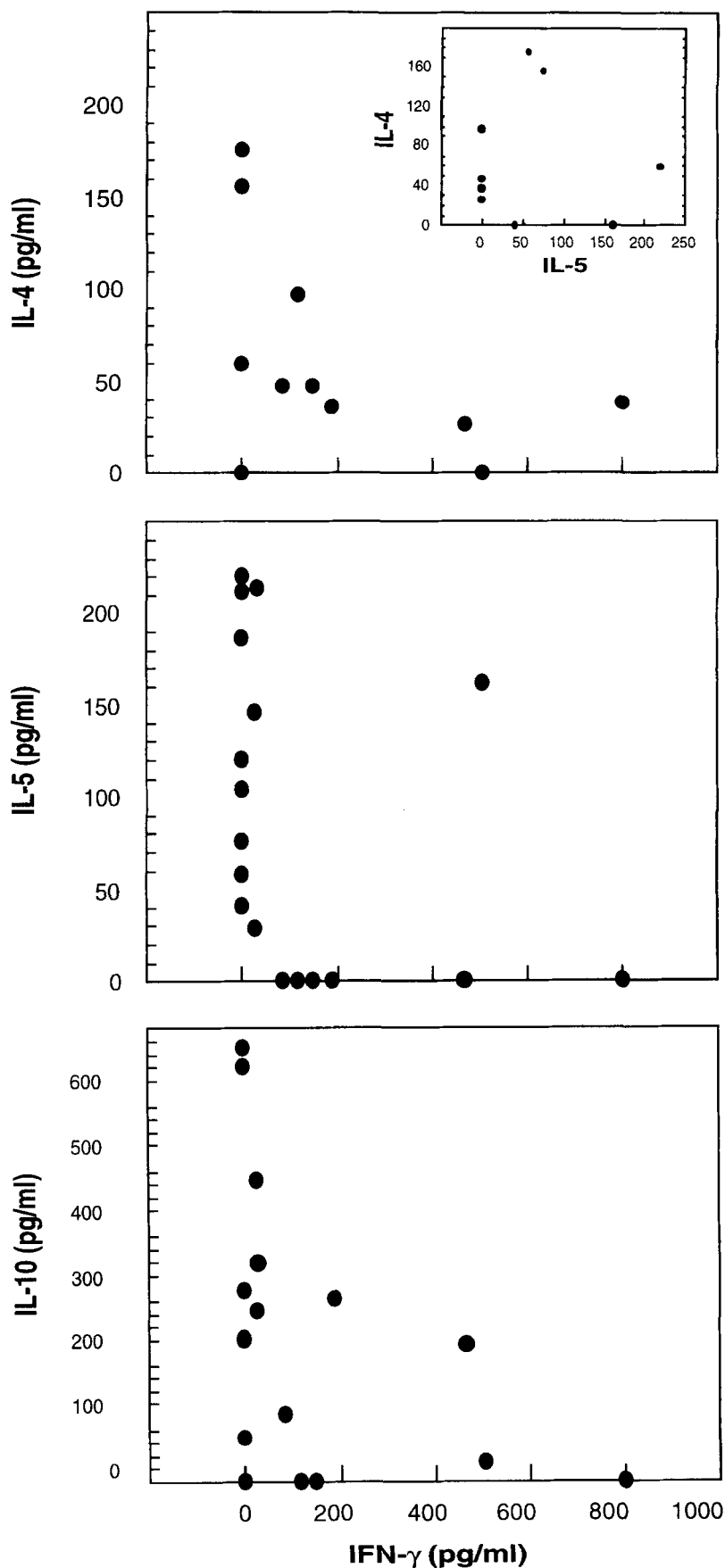
Discussion

The present study has identified several recombinant filarial Ags containing major B cell and T cell epitopes. These Ags direct IgG4 and IgE Ab secretion both in vivo and in vitro in filaria-infected patients in an IL-4- and an IL-13-dependent fashion.

Although it is generally thought that a relatively stable relationship exists between the so-called Th1 and Th2 CD4⁺ human T cell populations, in part through the production of counter-regulatory cytokines that suppress the function and the growth of the opposite cellular subset (15), CD4⁺ subset reorientation or bias by Ags occurs in certain pathologic situations (16). Indeed, it has been shown that chronic helminth infections bias CD4⁺ subset development toward Th2-type responses, which may be causally linked to the immediate hypersensitivity responses associated with these infections (1, 2, 17). Tissue-invasive helminth infections are commonly associated with high serum levels of polyclonal and Ag-specific IgG4 and IgE Abs, responses felt to be mediated by the Th2-type cytokines IL-4 and IL-13 and, perhaps, by the absence of the Th1-type cytokine IFN- γ (18, 19).

Evidence from many laboratories has indicated that induction of IgE synthesis requires two signals: one delivered by the cytokines IL-4 and IL-13, and one delivered by a B cell activating factor. This latter “second” signal is either delivered directly by T cells (through “cognate interactions”) or by T cell-independent factors such as EBV and hydrocortisone (20). Normal human B cells can generally undergo a (polyclonal) $\mu \rightarrow \epsilon$ switch upon IL-4 stimulation in the presence of either T cells, T cell membranes, or surrogate ligands for B cell molecules that are engaged during B cell activation such as CD40L (21–24). Activated human B cells may undergo a $\mu \rightarrow \gamma 4 \rightarrow \epsilon$ switch in vivo in response to any relevant allergen or to helminth parasite Ag, although the defined Ag/allergen-driven systems have been less well studied than the polyclonally induced ones. Indeed, few studies have focused on the role of IL-4 (IL-13) in Ag-specific systems, and even fewer in systems using molecularly defined Ags. In parasite systems, most of the studies done to date have been conducted using crude parasite Ag preparations that contain multiple B and T cell epitopes. Despite this, crude filarial Ag preparations have been shown to be capable of inducing IgE and IgG4 Ab secretion in vitro at extremely low concentrations (25–27). These Ag-induced responses appear to be reciprocally dependent on the frequencies of IL-4- and IFN- γ -secreting cell subsets (13, 28–31), although the mechanisms for CD4⁺ subset expansion awaits clarification. Although one such purified filarial Ag has been shown to drive IgE Ab

FIGURE 6. The relationship between IL-4, IL-5, or IL-10 induced by Ov27 and OvD5B to the production of IFN- γ . The levels of IL-4 (*top panel*), IL-5 (*middle panel*), or IL-10 (*bottom panel*) measured in culture supernatants of PBMCs stimulated with Ov27 or OvD5B are plotted against the levels of IFN- γ measured in the same cultures. In the inset (*top panel*) are plotted the levels of IL-4 against IL-5 measured in the same cultures.



production in an in vitro system (32), more work is needed to assess the issue of the structural basis of an allergic response and of the roles Ags (allergens) themselves play.

The present study identified two recombinant filarial proteins that induced IgG4 and IgE production in vitro using PBMCs from filarial parasite-infected patients in the absence of exogenous cytokines. Although this has not been analyzed at the single-cell level, our data suggest that the Ag-responsive B cells (in the context of T cells and monocytes/macrophages) have undergone a $\mu \rightarrow \gamma 4 \rightarrow \epsilon$ switching program for Ig secretion, a process that probably depends on IL-4 and IL-13. Indeed, this IgE/IgG4 production stimulated by Ag could be blocked by neutralizing anti-IL-4 or anti-IL-13 Abs or by sIL-4Rs. The results shown here suggest that there can be either a direct role for Th2-type cytokines (such as IL-4 and IL-13) or that B cells acquire differential responsiveness to Th2-type T cell cytokines (IL-4 and IL-13). Indeed, although our results are not in full agreement with data obtained in a murine system (33) in which a strict T-B cognate interaction for Ag-specific IgE responses in primed B cells was necessary and independent of IL-4, they do not exclude T-B cognate interactions as an important mechanism.

Central to the development of T cell responses—and, more precisely, T cell cytokine production—are counter-regulatory cytokines (e.g., IL-4 and IFN- γ ; 34). IFN- γ is the widely accepted antagonist for IL-4-mediated activities on B cell transcription (22, 35, 36). IL-12 has also been shown to down-regulate IgE production indirectly by promoting the expansion of Th1 cells and the secretion of IFN- γ and by inhibiting the development of IL-4-producing cells (37, 38). IL-12 also acts directly by regulating the CD28/B7 Ag interactions on the surface of T and B cells (39) and by suppressing the synthesis of IL-4-induced IgE (40). In addition, it has been recently shown that IL-12 is able to induce IFN- γ transcription and protein synthesis but not to down-regulate IL-4 transcription and synthesis by allergen-specific human Th2 clones (41). This suggests that IL-12 acts by inducing Th2 cells to switch to a Th0-type rather than to a Th1-type profile of cytokine secretion. In the present study, we have shown that the recombinant filarial proteins (Ags) OvD5B and Ov27 are capable of inducing patient PBMCs to produce IL-4, IL-5, and IL-10 (in contrast to IFN- γ). In a previous study, it was shown that CD4⁺ T lymphocytes from normal individuals and from helminth-infected patients have a very low frequency of cells producing both IL-5 and IFN- γ , a distinct subset producing both IL-4 and IFN- γ , but have higher frequencies of cells producing IL-4, IL-5, or IFN- γ alone. This phenomenon, echoing the Th0/Th1/Th2 paradigm, has been shown to help redefine Th0-, Th1-, and Th2-secreting cells (7). Consistent with these findings, this study suggests that Ov27 and OvD5B expand differentially those cells secreting IL-4, IL-5, or IL-10 (Th2) vs IFN- γ (Th1) or IL-4 (and occasionally IL-10) and IFN- γ (Th0).

Central to the present studies is an understanding of how and why helminth Ags stimulate so-called Th2 (or at least IL-4-mediated) responses. Finkelman and co-workers have hypothesized that proteolytic enzymes, that are produced in large quantities by certain parasites to allow them to penetrate the target tissues, might be responsible for Th2-type responses (42). Indeed, the injection of mice with papain, a cysteine protease molecule that favors the development of experimental IgE-mediated allergic responses, induced an increase of IL-4 gene expression as well as subsequent IgG1 (the mouse equivalent of human IgG4) and IgE Ab responses (42). This suggested that proteases might be stimuli for Th2 responses. Nevertheless, the major *Leishmania major* Ag, gp63 (a metalloprotease; 43), clearly elicits a Th1-type response in C57/Bl6 mice (44). Thus, other mechanisms must also be considered, including the duration or site of Ag exposure or the role the different types of APCs may play in eliciting Th1 vs Th2 responses (45–48). Interestingly, having selected among 24 available recombinant filarial Ags, the two Ags that were able to induce consistent in vitro IgE and/or IgG4 Ab production were not proteases but protease inhibitors (Ov27 being a cysteine protease inhibitor, analogous to Ov7/cystatin (49) and OvD5B (50) being an aspartyl protease inhibitor, analogous to Ov33 (GenBank X13313) (51, 52)). It has been suggested that protease inhibitors may interfere with Ag processing (18) and therefore prevent costimulation; it is also possible that these particular molecules share similarities with other allergens (52, 53), although motifs common among allergens have not yet been identified. By mapping specific IgE epitopes of allergens, it may be possible to explore this possibility further.

The regulation of immediate hypersensitivity-type responses in filarial infections must be considered to result from an interplay among many different cytokines. In that helminth parasites reproducibly induce such characteristic immune responses, the use of defined rAgs and in vitro Ab production should provide insights not only into the regulation of such reactions but also to an understanding of how these can be modulated.

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