INFECTION WITH *NIPPOSTRONGYLUS BRASILIENSIS* OR INJECTION OF ANTI-IgD ANTIBODIES MARKEDLY ENHANCES Fc-RECEPTOR-MEDIATED INTERLEUKIN 4 PRODUCTION BY NON-B, NON-T CELLS

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Lymphokines (LK)[1] are polypeptides that mediate many of the regulatory functions of the immune system. Among these are a set of molecules that are produced by T cells in response to receptor-mediated stimuli and act upon B lymphocytes, macrophages, other cells of hematopoietic lineage, and on the T cells themselves (1). It has recently been demonstrated that crosslinkage of Fc receptors on growth factor–dependent mast cell and myeloid cell lines stimulates the production of some of these "T cell–derived" molecules, including IL-4, IL-5, IL-3, and granulocyte/macrophage colony-stimulating factor (GM-CSF) (2–5). Non-transformed mast cell lines have also been reported to secrete IL-6, IL-1, TNF-α and several of the macrophage inflammatory proteins in response to cross-linkage of high affinity Fcϵ receptors (3, 5).

We have recently shown that a population of spleen cells that lack both B cell and T cell markers and do not express the NK cell marker asialo-GM1 on their surface produce IL-4 in response to incubation on culture dishes coated with IgE (6). The response of these cells to plate-bound (PB) IgE is inhibited by low concentrations of soluble IgE, strongly suggesting that it is mediated by cross-linkage of a high affinity Fcϵ receptor. The production of IL-4 by splenic non-B, non-T cells from untreated mice in response to PB-IgE is markedly enhanced by the presence of IL-3. In the presence of IL-3, PB-IgG1, IgG2a, and IgG2b are also potent stimuli of IL-4 production by splenic non-B, non-T cells from naive animals.

In this report, we examine the changes in the LK-producing capacity of the non-B, non-T cell population in mice that have been injected with anti-IgD antibodies as well as in mice that have been infected with the helminthic parasite *Nippostrongylus*

brasiliensis (Nb). Both anti-IgD injection and infection with Nb elicit an immune response marked by production of large amounts of polyclonal IgE (7, 8). We chose these immunization protocols, which cause IgE to be formed in large amounts, because it has been shown that IL-4 is essential for in vivo IgE responses (9) and because increased IL-4 production has been reported in one of these situations (10).

We show here that mice that have received anti-IgD antibodies or have been infected with Nb exhibit a considerable increase in the number of splenic non-B, non-T cells. Furthermore, non-B, non-T cells from mice that have received injections with anti-IgD or have been infected with Nb produce much more IL-4 in response to PB-IgE or PB-IgG2a than do such cells from normal mice and can do so without need for the addition of IL-3. These striking changes in IL-4-producing non-B, non-T cells suggest that such cells may play a physiologic role in responses to agents that elicit immune responses marked by elevation of serum IgE levels.

Materials and Methods

Animals. Virus-free female BALB/c mice, 8–12 wk old, were obtained from Division of Cancer Therapy Animal Program, National Cancer Institute. Nb infection was performed by subcutaneous injection of 1,000 Nb larvae. Goat–mouse IgD antibodies (800 μg) were injected in the tail vein.

Culture Medium. RPMI 1640 (Biofluids, Rockville, MD) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), L-glutamine (2 mM), 2-ME (0.05 mM), Hepes (10 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) was used for culturing cells.

Preparation of Non-B, Non-T Cells; B Cells; and T Cells. Single cell suspensions were prepared from spleens, lymph nodes, or thymus in cold HBSS. Marrow cell suspensions were prepared by repeated flushing of femoral marrow cavities with cold HBSS through a 23-gauge needle. Single cell suspensions from lungs were prepared by enzymatic digestion of washed lungs by three cycles of incubation each for 30 min at 37°C in culture medium containing 5% FCS, 100U/ml collagenase (Type II; Sigma Chemical Co., St. Louis, MO), 30 U/ml DNase (Type I; Sigma Chemical Co., St. Louis, MO), 0.05% soybean trypsin inhibitor. The lung cell suspensions were fractionated on Percoll density gradients (Pharmacia Fine Chemicals, Uppsala, Sweden). The cells were layered onto discontinuous gradient (70, 66, 60, and 50% Percoll) and centrifuged at 1,000 *g* for 20 min at room temperature. The cells in the interphase between 50 and 60% Percoll were washed twice with cold HBSS and used as lung cell suspensions.

Red blood cells (from spleen and bone marrow) were lysed by suspension of cells in ammonium chloride-potassium carbonate lysing buffer. Nucleated cells were washed 2× in HBSS and suspended in culture medium containing 5 mM EDTA (staining buffer). Depletion of T and B cells, that had been stained with a mixture of FITC-labeled anti-T and anti-B reagents, with magnetic beads coated with sheep anti-FITC, was performed as described previously (6). The FITC-labeled antibodies used were anti-Thy-1.2 (30H12), anti-CD3 (2C11), anti-CD4 (Gk1.5), anti-CD8 (2.43), anti-B220 (6B2), and anti-Ia^d (MKD6). Efficiency of depletion was determined by cytometric analysis using a FACScan (Becton Dickinson & Co., Mountain View, CA).

Lymphokines and Cytokines. Human rIL-2 was a gift of Cetus Corp. (Emeryville, CA). IL-2 activity was defined in terms of "Cetus Units." 1 Cetus Unit is equivalent to 6 WHO international units. Synthetic IL-3 was a gift of Dr. Ian Clark-Lewis (Biomedical Research Center, Vancouver, Canada). 1 U of IL-3 was defined as the amount of IL-3 that stimulated half maximal [^{3}H]thymidine incorporation by FDC/1 cells. Mouse rIL-4 was obtained from a baculovirus expression system, using a vector into which the IL-4 gene had been inserted by C. Watson, Laboratory of Immunology, NIAID. IL-4 activity was measured using the CT.4S cell line (11), comparing it with a standard that had initially been calibrated on the basis of 10 U/ml being equal to the amount of IL-4 required for half maximal stimulation

of [³H]thymidine uptake by resting B cells stimulated with 5 µg/ml of goat anti-IgM antibody (12).

Immunoglobulins and Monoclonal Antibodies. Purified mouse IgG1, IgG2a, and IgG2b were purchased from Southern Biotechnology (Birmingham, AL). Mouse IgE anti-DNP mAb (HI-DNP-ε-26) (13) was purified from ascites fluid by ammonium sulfate precipitation, DE52 ion-exchange chromatography and gel filtration (14). Purified monoclonal rat anti-mouse IL-4 (11B11) (15) was prepared by Verax Corp. (Hanover, NH). (Fab′)₂ goat anti-mouse IgG Fc was purchased from Organon-Cappel (West Chester, PA). (Fab′)₂ anti-mouse IgE was prepared by pepsin digestion of monoclonal rat anti-IgE (B1E3) followed by purification by gel filtration.

The following antibodies were prepared by a combination of ammonium sulfate precipitation, DE52 ion-exchange chromatography, and Sephadex G200 gel filtration (16); anti-Thy-1.2 (30H12) (17); anti-CD3 (2C11) (18); anti-CD4 (Gk1.5) (19); anti-CD-8 (2.43) (20); anti-B220 (6B2) (21); and anti-Ia^d (MKD6) (22). Purified Ig was fluoresceinated with FITC, as previously described (23). Affinity purified goat anti-mouse IgD antibody was prepared as described elsewhere (16).

Measurement of Il-4 Production from Cells Stimulated by PB Antibodies. Non-B, non-T cells were cultured for 36 h in 96-well microtiter Immulon-2 plates that had been coated with 10 µg/ml of the indicated Ig. After irradiation (1,000 rad), 5000 CT.4S cells (11) were added per well and IL-4 production was determined by an 8-h pulse with [³H]thymidine 48 h later. Anti-IL-2 mAb (11B11) completely inhibits [³H]thymidine uptake by CT.4S cells that have been cocultured with non-B, non-T cells stimulated with PB-Ig. This indicates that IL-4 was the lymphokine whose production was measured by this assay. All determinations were performed in triplicate; relative standard errors had a mean of <15%. IL-4 production is shown as either direct cpm incorporated or IL-4 equivalent units, determined from a standard curve performed with the CT-4S cells in the same assay using known concentrations of added rIL-4.

Nb Antigen. Secretory antigen from adult Nb worms was isolated as described by Jarboe et al. (24). Briefly, adults worms were rinsed out of the upper one-third of the small intestine of rats injected 12 d previously with 3,000 Nb larvae. The worms were washed with RPMI 1640, placed in petri dishes in RPMI 1640 with antibiotics (penicillin, streptomycin, and gentamicin) and incubated for 48 h. The supernatant was then filtered and the protein content determined by the Bradford assay.

Results

Injection of Anti-IgD and Infection with Nb Increases the Number of Splenic Non-B, Non-T Cells and Their Production of IL-4. The spleens of normal mice contain a population of cells that lack the B and T cell markers Thy1, CD3, CD4, CD8, Ia, and B220 (Fig. 1). In mice that have been injected with anti-IgD antibodies 5 d earlier or infected with Nb larvae 9 d earlier, there is a striking increase in the fraction of spleen cells in the non-T category. In the experiments illustrated in Fig. 1, 20.9% of spleen cells from Nb-infected and 19.8% of spleen cells from anti–IgD-injected mice are non-B, non-T cells while the controls (spleen cells from naive mice) for these experiments were 6.4 and 2.5%.

Non-B, non-T cells were purified from spleens of normal BALB/c mice and from spleens of anti-IgD-injected and Nb-infected BALB/c mice through the use of anti-FITC–conjugated to magnetic beads to deplete cells that had bound FITC antibodies specific for the markers listed in the paragraph above. To test the IL-4-producing capacity of non-B, non-T cells from these mice, varying numbers of cells were cultured in 96-well dishes to which IgE had been adsorbed. Since non-B, non-T cells from naive mice require the presence of IL-3 for optimal IL-4 production in response to PB-IgE (5), IL-3 was added to all cultures. As shown in Fig. 2, 3 × 10³
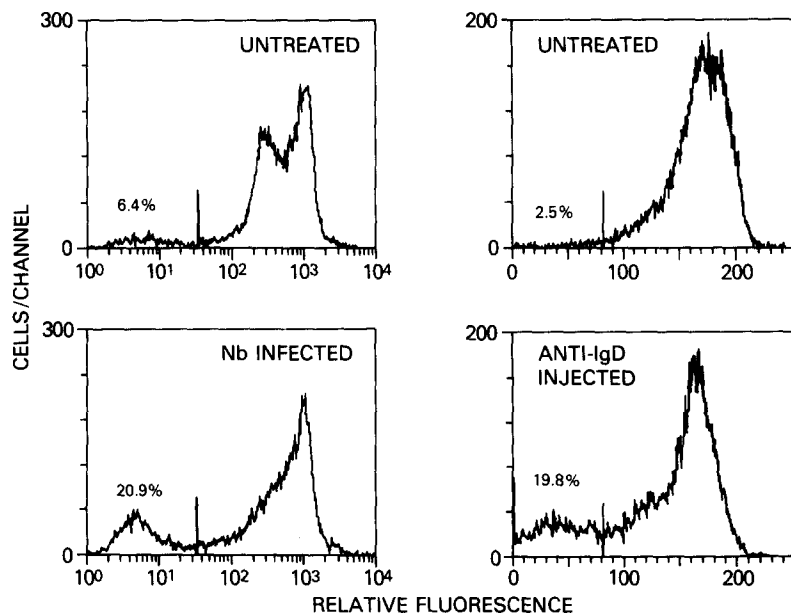
Figure 1.   Nb infection and injection of anti-IgD markedly increase the frequency of splenic non-B, non-T cells. Spleen cells from Nb-infected mice and from anti-IgD-injected mice were compared with normal control animals (untreated) for frequency of non-B, non-T cells by staining with FITC-conjugated anti-Thy-1, CD3, CD4, CD8, B220, and Ia antibodies and examined by FACS analysis. The percentages shown refer to cells not stained by these antibodies. The histograms shown here reflect two separate experiments. The results for cells from the Nb infected and untreated mice (left histograms) ..re reported as relative fluorescence intensity, while those for cells from anti-IgD-injected and untreated mice (right histograms) are reported as fluorescence channel number. In both cases, the data were collected using logarithmic amplification.

non-B, non-T cells from Nb-infected mice make an amount of IL-4 comparable to that made by 10^5 non-B, non-T cells from normal mice. A similar amount of IL-4 is made by 10^4 non-B, non-T cells from anti-IgD-injected mice. These results thus indicate a striking increase in the capacity of non-B, non-T cells from both groups of mice to produce IL-4.

To be certain that IL-4 was produced by non-B, non-T cells, we harvested cells at the end of the culture period and stained them with anti-B220, CD-3 and anti-Thy-1. The cells failed to stain with these reagents (data not shown) indicating that the responses of the non-B, non-T cell population could not be accounted for by a small number of either B or T cells that had not been eliminated in the initial purification and had been expanded during the culture period. Furthermore, we have previously shown (6) that neither B cells nor T cells prepared from the same starting population as the non-B, non-T cells produced IL-4 in response to PB-Igs, indicating that non-B, non-T cells were the sole population in the spleen responsible for IL-4 production in response to Fc receptor crosslinkage.

We have evaluated total numbers of spleen cells and percentages of non-B, non-T cells from normal and anti-IgD-injected mice in a series of experiments. Thus, anti-IgD-injected mice yield 1.09 (± 0.21 SE) × 10^8 cells per spleen while the mean
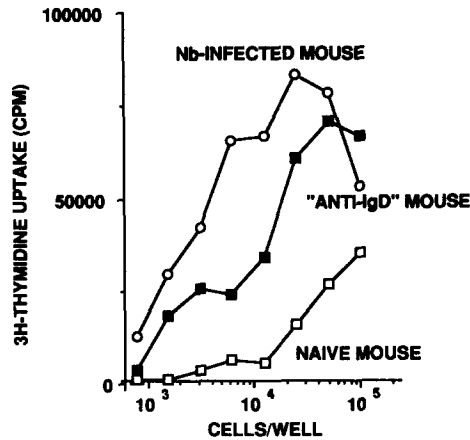
FIGURE 2. IL-4 production by non-B, non-T cells is enhanced by Nb-infection or anti–IgD injection. Non-B, non-T cells from spleens of naive, goat anti–IgD-injected (5 d earlier) or Nb-infected (9 d earlier) mice were incubated at various cell densities on IgE-coated wells (10 μg/ml) in the presence of IL-3 (50 U/ml). After 36 h, the cells were irradiated (1,000 rad) and CT.4S cells were added (5,000 cells/well). [^{3}H]Thymidine was added 48 h later and incorporation into the DNA was determined after 10 h.

number of spleen cells obtained from naive BALB/c mice was 0.41 (± 0.03) × 10^8. The percentage of non-B, non-T cells in anti–IgD-injected spleens was 13.3 (± 1.6), while in normal spleen cell populations it was 7.8 (± 1.0). Thus anti-IgD mice have 4.7-fold more splenic non-B, non-T cells than do normal mice. Since these cells are ∼10-fold better on a per cell basis in producing IL-4, the overall increase in these mice is on the order of 50-fold. Although similar comparisons have not been performed with for Nb-infected mice, the absolute increase in the size of the splenic non-B, non-T cell population is at least as great as in the anti–IgD mice, and as shown above, the increase in production per cell is even larger, indicating a greater increase in IL-4-producing capacity of spleen cells from these mice.

Not only are non-B, non-T cells more numerous in the spleens of Nb-infected and anti–IgD-injected mice, these cells can also produce substantial amounts of IL-4 in the absence of IL-3 (Fig. 3). The addition of IL-3 further increases the IL-4 producing capacity of these cells but their dependence on IL-3 is much less dramatic than is the case for IL-4 production by non-B, non-T cells from naive mice in response to PB-IgE.

Non-B, Non-T Cells from Nb-infected or from Anti–IgD-injected Mice Produce IL-4 in Response to Anti-Ig Antibodies or to Antigen. Non-B, non-T cells from mice infected with Nb larvae 9 d earlier produced IL-4 when cultured on dishes to which an Nb antigen-containing extract had been adsorbed (Fig. 4). By contrast, non-B, non-T cells from naive mice, even in the presence of IL-3, failed to produce IL-4 in response to Nb antigen. This strongly suggests that Fc receptors on non-B, non-T cells from Nb-infected mice have bound antibody specific for Nb antigen and crosslinkage of these receptors by antigen leads to stimulation of lymphokine production.

To further examine the role of Ig bound to non-B, non-T cells as a target for crosslinkage and subsequent stimulation of IL-4 production, we examined the capacity of F(ab')$_2$ fragments of heavy chain-specific anti–IgE and anti–IgG antibodies to simulate IL-4 production by cells from mice that had not been injected with anti-IgD (day 0 mice) or at 6 and 16 d after injection (Fig. 5). In the absence of IL-3, non-B, non-T cells (12,500/well) from day 0 mice failed to produce detectable amounts of IL-4 in response to PB-IgE or PB-IgG2a. PB anti-IgE also failed to stimulate
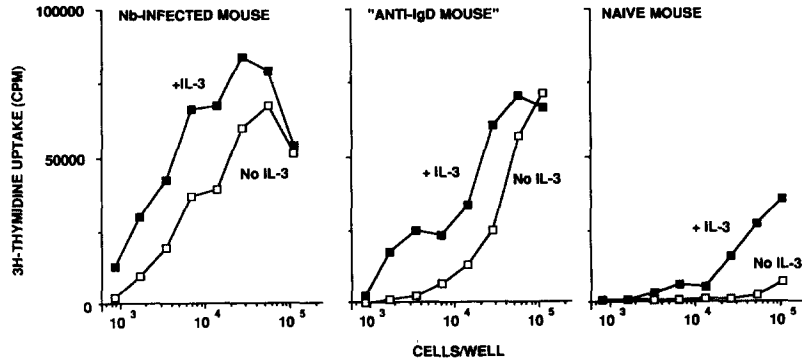
FIGURE 3.   Non-B, non-T cells from Nb-injected or anti–IgD-injected mice produce substantial amounts of IL-4 in the absence of IL-3. Non-B, non-T cells from spleens of naive, goat anti–IgD-injected (5 d earlier) or Nb-injected mice (9 d earlier) were incubated at various cell densities on IgE-coated wells (10 μg/ml) in the presence or absence of IL-3 (50 U/ml). (These are the same cells as were used in the experiment illustrated in Fig. 2.) IL-4 production was measured by co-culture with CT.4S cells as described in the legend to Fig. 2.

a response while PB anti-IgG did elicit production of modest levels of IL-4. In the presence of IL-3, all the stimulants elicited production of some IL-4 from non-B, non-T cells from naive mice. Anti-IgE was a poorer stimulant than anti-IgG. However, injection of anti-IgD caused a substantial increase in IL-4 production in response to anti-IgE, which was noted on both days 6 and 16. There was also an increase in IL-4 production in response to anti-IgG. These results are consistent with the striking increases in IgE and IgG1 levels that occur in anti–IgD-injected mice beginning at about 6 d and indicate that Ig bound to non-B, non-T cells in vivo can act as a "receptor" for stimulation of IL-4 production, presumably by the cross-linkage of Fc_ϵ and of Fc_γ receptors to which the Igs were bound.

It should also be noted that some IL-4 is produced by non-B, non-T cells from Nb-infected (Fig. 4) and anti–IgD-injected mice (Fig. 5) in response to IL-3 alone. The mechanism for this has not been determined, but might reflect the enhance-
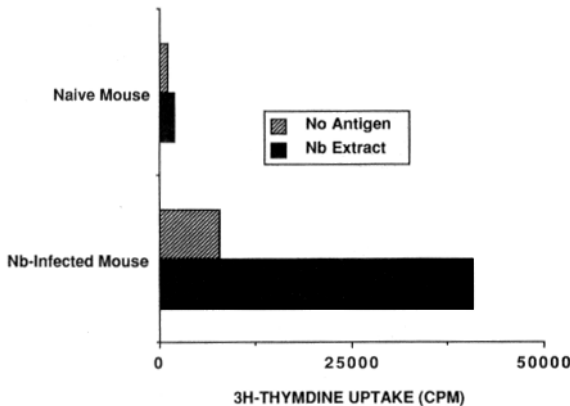

FIGURE 4.   Nippostrongylus antigen triggers IL-4 production by non-B, non-T cells from Nb-infected mice. Non-B, non-T cells (20,000/well) from the spleens of naive or Nb-infected (9 d earlier) mice were cultured in the presence of IL-3 on microtiter wells that had been coated with nothing or Nb extract (10 μg/ml). IL-4 production was measured by co-culture with CT.4S cells as described in the legend to Fig. 2.
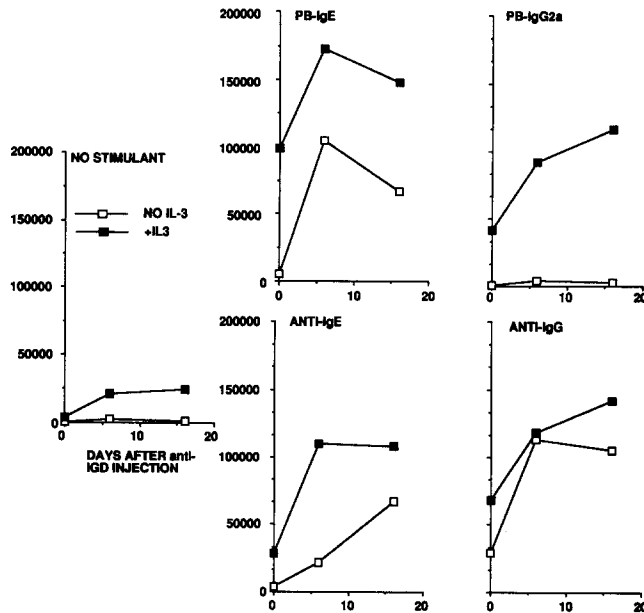
FIGURE 5. PB-Ig and anti-Ig induces IL-4 production by non-B, non-T cells at various times after anti-IgD injection. Non-B, non-T cells from the spleens of naive or anti IgD-injected mice (6 or 16 d earlier) mice were incubated in culture medium with or without IL-3 on microtiter wells (12,500 cells/well) that had been coated with nothing, IgE, IgG2a, (Fab')2 goat anti-mouse IgG Fc or (Fab')2 rat anti-mouse IgE (10 μg/ml). IL-4 production was measured by co-culture with CT.4S cells as described in the legend to Fig. 2.

ment by IL-3 of a "weak" crosslinking of Fc$_\gamma$ or Fc$_\epsilon$ receptors caused by the presence of some immune complexes bound, in vivo, to these receptors.

Organ Distribution of IL-4 Producing Non-B, Non-T Cells from Naive and Nb-infected Mice.   Non-B, non-T cells were prepared from spleen, bone marrow, thymus, and mesenteric lymph node of untreated mice. PB-IgE, in the presence of IL-3, caused considerable IL-4 production by splenic non-B, non-T cells. Bone marrow cells produced even more IL-4 in response to PB-IgE and to PB-IgG2a than did splenic non-B, non-T cells (Fig. 6). By contrast, no production of IL-4 in response to PB-Ig was obtained from mesenteric lymph node or thymus non-B, non-T cells. In other experiments (not shown), peritoneal non-B, non-T cells also failed to produce IL-4 in response to PB-Ig in the presence of IL-3.

Anti-IgD-injected and Nb-infected mice showed striking production of IL-4 from splenic and bone marrow non-B, non-T cells (data not shown). However, even in these mice non-B, non-T cells prepared from lymph node, thymus, or peritoneum did not produce IL-4 in response to PB-Ig. Since Nb larvae migrate through the lung before maturing into adult worms in the intestinal tract of the mouse, we examined the IL-4 producing capacity of non-B, non-T cells in the lungs of Nb-infected mice. These cells produced considerable amounts of IL-4 in response to PB-IgE, PB-IgG2a or PB F(ab')2 anti-IgG while lung non-B, non-T cells from normal or anti-IgD-injected mice did not respond to these stimulants (Fig. 7). It should be noted that, IL-3 alone stimulated IL-4 production from lung non-B, non-T cells from Nb-infected mice.

Discussion

The capacity of transformed mast cells to constitutively produce IL-4 (25) and of nontransformed mast cell lines to produce IL-4 and other lymphokines in response
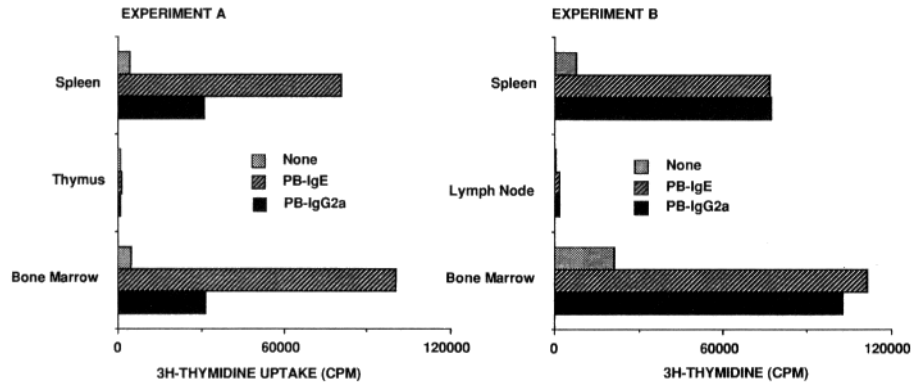
FIGURE 6.   Non-B, non-T cells from bone marrow but not thymus or lymph nodes produce IL-4 in response to PB-Ig. Non-B, non-T cells from the spleens, lymph nodes, thymus, or bone marrow of naive mice were incubated in culture medium containing IL-3 (50 U/ml) on wells (25,000 cells/well) that had been coated with nothing, IgG2a, or IgE (10 μg/ml). IL-4 production was measured by co-culture with CT.4S as described in the legend to Fig. 2.

to Fcε receptor crosslinkage (3–5) raised the possibility that normal counterparts of these cells might be important physiologic producers of IL-4. Other previous results indicated that T-depleted splenocytes from Nb infected mice produced IL-4, as determined by maintenance of FcεRII levels and that the optimum IL-4 production was seen on days 9–11 post-Nb infection (26). Recently, using a more sensitive assay, we demonstrated that a spleen cell population lacking both T cell and B cell markers produced substantial amounts of IL-4 in response to stimulation with PB-IgE (6). This response appears to be mediated by crosslinkage of FcεR1, and when cells are obtained from naive mice, is markedly enhanced by treatment of cells with IL-3. These cells are not mature mast cells, since mature mast cells are not found in the non-B, non-T cell population obtained from spleens of naive mice. We have suggested that mast cell precursors may be the cell population mediating this effect, but definitive evidence establishing this has not been obtained. The organ distribution of IL-4-producing non-B, non-T cells (spleen and bone marrow but not lymph
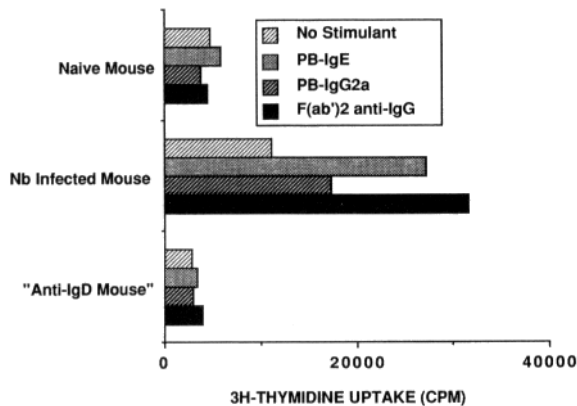

FIGURE 7.   Non-B, non-T cells in lungs of Nb-infected mice produce IL-4 in response to FcR crosslinkage. Non-B, non-T cells from the lungs of naive, goat anti-IgD-injected (5 d earlier) or Nb-infected (9 d earlier) mice were incubated in medium containing IL-3 (50 U/ml) on microtiter wells (20,000 cells/well) that had been coated with nothing, IgE, IgG2a, or (Fab')₂ goat anti mouse IgG (10 μg/ml). IL-4 production was measured by co-culture with CT.4S cells as described in the legend to Fig. 2.

node, thymus, Peyer's patch, or peritoneum) is consistent with the cell being a he-matopoietic progenitor cell.

IL-4 production has also been shown to be mediated by stimulation with PB-Ig's of the IgG2a, IgG1, and IgG2b classes (6). Both Nb-infected and anti–IgD-injected mice show striking enhancement in their production of IL-4 in response to both PB-IgE and PB-IgG2a. Although it is not clear whether the cells that produce IL-4 in response to PB-IgG are the same as those that respond to PB-IgE, the receptors through which the effects are mediated are distinct. Thus, soluble IgE is an effective inhibitor of IL-4 production in response to PB-IgE but not in response to PB-IgG2a while the monoclonal anti-FcγR2 antibody 2.4G2, inhibits IL-4 production in re-sponse to PB-IgG2a but not to PB-IgE (6).

The current studies show that in two situations in which there are striking in-creases in serum IgE levels, there are parallel increases both in the numbers of spleen cells that lack T cell and B cell markers, and in the amount of IL-4 produced by a standard number of these non-B, non-T cells. Thus, in mice injected with anti-IgD antibodies and in animals infected with Nb larvae, 10–33-fold fewer non-B, non-T cells are required for the production of a standard amount of IL-4 than is the case for non-B, non-T cells from normal mice. Furthermore, spleens from anti–IgD-injected mice contain more than four times as many non-B, non-T cells as do spleens from naive mice.

In addition to an increase in the IL-4-producing capacity of the splenic non-B, non-T cell population of Nb-infected and and of anti–IgD-injected mice, these cells produce considerable amounts of IL-4 without a requirement for addition of IL-3, whereas cells from naive animals are strikingly dependent upon the addition of IL-3 to the culture.

IL-4 production by cells from Nb-infected and anti–IgD-injected mice can be elicited not only by PB-IgE but also by PB-F(ab')2 fragments of anti-IgE antibodies, sug-gesting that the Fcε receptors of these mice have bound IgE in vivo and thus can be indirectly crosslinked by anti-IgE in vitro. Similarly, we observed that splenic non-B, non-T cells from Nb-infected mice can be stimulated to produce IL-4 by an antigen extract derived from Nb worms. This indicates that antigen can crosslink specific antibodies bound to Fc receptors on non-B, non-T cells and lead to IL-4 production. In this situation, we have not determined whether the antibody in ques-tion is cell-associated IgG or IgE. It should be noted that anti-IgG antibodies are also excellent stimulants of IL-4 production by non-B, non-T cells and that this stim-ulant acts efficiently both on cells from naive donors and from immune donors. These results indicate that antigen as well as antigen–antibody complexes could be in vivo stimulants of IL-4 production by non-B, non-T cells, providing a potential physio-logical means for lymphokine production by these cells.

One unexplained observation is that non-B, non-T cells in immune mice produce measurable amounts of IL-4 in response to treatment with IL-3 only. One possi-bility is that these cells have bound IgE or IgG that is minimally crosslinked providing a signal too modest to elicit IL-4 production without the presence of added IL-3. Alternatively, IL-3 may, under certain conditions, directly stimulate lymphokine production from "activated" non-B, non-T cells.

Overall, these experiments indicate that the IL-4 producing capacity of non-B, non-T cells expands dramatically in Nb infection and in association with anti-IgD

injection. This strongly suggests that these cells have an important role to play in the regulation of responses associated with these forms of immunization. Furthermore, the fact that the cells become less IL-3-dependent after immunization than was the case for non-B, non-T cells from naive animals could be explained by the action of IL-3 upon these cells in vivo. We (LeGros, G., S. Z. Ben-Sasson, D. H. Conrad, I. Clark-Young, M. Plaut, F. D. Finkelman, and W. E. Paul, manuscript in preparation) have observed that IL-3 injection into mice increases IL-4 production from peritoneal and splenic non-B, non-T cell populations. If IL-3 is required in vivo for the expansion and relative IL-3 independence of immune non-B, non-T cells, this would suggest that the immunization effects observed might result from T cell production of IL-3. Thus, the production of lymphokines by non-B, non-T cells might very well reflect a means through which T cell function could be strikingly amplified in vivo by recruitment of a "new" IL-4-producing cell population.

Summary

Non-B, non-T cells from spleen and bone marrow of naive mice produce IL-4 upon stimulation by plate-bound IgE or IgG2a in the presence of IL-3. Infection of mice with Nippostrongylus brasiliensis (Nb) or injection of anti-IgD antibodies, treatments known to cause striking polyclonal IgE responses, increase the number of splenic non-B, non-T cells and cause 10–30-fold increase in IL-4 production by a standard number of these cells. In Nb-infected mice, IL-4 producing non-B, non-T cells can be found in the lungs, a site through which Nb larvae migrate. Non-B, non-T cells from anti–IgD-injected mice produce IL-4 in response to anti-IgE antibodies, indicating that these cells have been sensitized in vivo with IgE and that crosslinkage of such IgE can lead to stimulation of lymphokine production. Similarly, non-B, non-T cells from Nb-infected mice produce IL-4 upon stimulation with Nb-antigen, indicating that antigen can also crosslink receptors on in vivo sensitized non-B, non-T cells and stimulate lymphokine production. The striking increases in the IL-4-producing capacity of the splenic non-B, non-T cell population in anti–IgD-injected and Nb-infected mice and the in vivo sensitization of these cells strongly suggests that they may have an important role in lymphokine production in helminthic infections and other situations marked by striking elevations of serum IgE levels.

References

1. Paul, W. E. 1989. Pleiotropy and redundancy: T cell-derived lymphokines in the immune response. *Cell.* 57:521.
2. Le Gros, G., J. Le Gros, and J. D. Watson. 1987. The induction of lymphokine synthesis and cell growth in IL3-dependent cell lines using Ag.Ab complexes. *J. Immunol.* 134: 422–428.
3. Plaut, M., J. H. Pierce, C. J. Watson, J. Hanley Hyde, R. P. Nordan, and W. E. Paul. 1989. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. *Nature (Lond.).* 339:64.

4. Wodnar-Filipowicz, A., C. H. Heusser, and C. Moroni. 1989. Production of the hae-
   mopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE
   receptor-mediated activation. *Nature (Lond.).* 339:150.

5. Burd, P. R., H. W. Rogers, J. R. Gordon, C. A. Martin, S. Jayaraman, S. D. Wilson,
   A. M. Dvorak, S. J. Galli, and M. E. Dorf. 1989. Interleukin 3-dependent and
   -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J.
   Exp. Med.* 170:245.

6. Ben-Sasson, S. Z., G. LeGros, D. H. Conrad, F. D. Finkelman, and W. E. Paul. 1990.
   Cross-linking Fc receptors stimulates splenic non-B, non-T cells to secrete IL-4 and other
   lymphokines. *Proc. Natl. Acad. Sci. USA.* In press.

7. Finkelman, F. D., C. M. Snapper, J. D. Mountz, and I. M. Katona. 1987. Polyclonal
   activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction
   of a polyclonal IgE response. *J. Immunol.* 138:2826.

8. Jarrett, E. E., and D. C. Stewart. 1972. Potentiation of rat reaginic (IgE) antibody by
   helminth infection. Simultaneous potentiation of separate reagins. *Immunology.* 23:749.

9. Finkelman, F. D., I. M. Katona, J. F. Urban, Jr., C. M. Snapper, J. Ohara, and W. E.
   Paul. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to
   the lymphokine B-cell stimulatory factor-1. *Proc. Natl. Acad. Sci. USA.* 83:9675.

10. Finkelman, F. D., J. Ohara, D. K. Goroff, J. Smith, N. Villacreses, J. J. Mond, and
    W. E. Paul. 1986. Production of BSF-1 during an in vivo, T-dependent immune response.
    J. Immunol. 137:2878.

11. Hu-Li, J., J. Ohara, C. Watson, W. Tsang, and W. E. Paul. 1989. Derivation of a T
    cell line that is highly responsive to IL-4 and IL-2 (CT.4R) and of an IL-2 hyporespon-
    sive mutant of that line (CT.4S). *J. Immunol.* 142:800.

12. Grabstein, K., J. Eisenman, D. Mochizuki, K. Shanebeck, P. Conlon, T. Hopp, C. March,
    and S. Gillis. 1986. Purification to homogeneity of B cell stimulating factor. A molecule
    that stimulates proliferation of multiple lymphokine-dependent cell lines. *J. Exp. Med.*
    163:1405.

13. Liu, F. T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman,
    N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenol specific murine IgE
    antibody preparation, isolation and characterization. *J. Immunol.* 124:2728.

14. Isersky, C., A. Kulczycki, Jr., and H. Metzger. 1974. Isolation of IgE from reaginic rat
    serum. *J. Immunol.* 112:1909.

15. Ohara, J., and W. E. Paul. 1985. Production of a monoclonal antibody to and molecular
    characterization of B-cell stimulatory factor-1. *Nature (Lond.).* 315:333.

16. Finkelman, F. D., S. W. Kessler, J. F. Mushinski, and M. Potter. 1981. IgD secreting
    murine plasmacytomas. Identification and partial characterization of two myeloma pro-
    teins. *J. Immunol.* 126:680.

17. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse
    lymphoid differentiation antigens. *Immunol. Rev.* 47:63.

18. Leo, O., M. Foo, D. Sachs, L. E. Samulson, and J. A. Bluestone. 1987. Identification
    of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA.*
    84:1374.

19. Dyalynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M.
    Pierres, and F. D. Fitch. 1983. Characterization of the murine T cell surface molecule
    designated L3T4, identified by the monoclonal antibody GK1.5. *J. Immunol.* 131:2445.

20. Sarmiento, M., A. L. Glasebrook, and F. W. Fitch. 1980. IgG or IgM monoclonal anti-
    bodies reactive with different determinants of the molecular complex bearing Lyt2 an-
    tigen block T cell mediated cytolysis in the absence of complement. *J. Immunol.* 125:2665.

21. Coffman, R. L. 1982. Surface antigen expression and immunoglobulin gene rearrange-
    ment during mouse Pre B development. *Immunology.* 69:5.

22. Kappler, J., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible H-2 re-

stricted interleukin-2 producing T cell hybridoma. Lack of independent antigen and H-2 recognition. *J. Exp. Med.* 153:1198.

23. Finkelman, F. D., and I. Scher. 1979. Rhesus monkey B lymphocyte surface immunoglobulin: analysis with a fluorescence activated cell sorter. *J. Immunol.* 122:1757.

24. Jarboe, D. L., J. S. Marshall, T. R. Randolf, A. Kukolja, and T. F. Huff. 1989. The mast-cell-committed progenitor. I. Description of a cell capable of IL-3-independent proliferation and differentiation without contact with fibroblasts. *J. Immunol.* 142:2405.

25. Brown, M. A., J. A. Pierce, C. J. Watson, J. Falco, J. H. Ihle, and W. E. Paul. 1987. B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. *Cell.* 50:809.

26. Rao, M., and D. H. Conrad. 1989. IL-4 production by T depleted cells from *Nippostrongylus brasiliensis* infected mice. *Immunol. Invest.* 18:1055.