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Immunology

Cross-linking Fc receptors stimulate splenic non-B, non-T cells to secrete interleukin 4 and other lymphokines

(interleukin 3/mast cell/Fcγ receptor II/Fcε receptor I/immunoglobulin)

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ABSTRACT **Spleen cell populations depleted of both B and T lymphocytes produce interleukin 4 (IL-4) in response to stimulation with immunoglobulins bound to the surface of culture dishes. In the presence of interleukin 3 (IL-3), plate-bound (PB) IgE and PB-IgG1, IgG2a, and IgG2b are excellent stimulants, whereas PB-IgA and PB-IgM fail to stimulate IL-4 production. In the absence of IL-3, PB-IgE stimulates relatively modest production of IL-4, whereas PB-IgG2a generally does not. The response to PB-IgE is inhibited by soluble IgE; antibody to Fcγ receptor II inhibits the response to PB-IgG2a. Thus, separate receptors mediate these stimulations, and Fc receptor cross-linkage is required for IL-4 production. Depletion of cells expressing asialo-GM1 does not diminish IL-4 production in response to PB immunoglobulins, indicating that natural killer cells are not essential for non-B, non-T cell production of IL-4. In addition to IL-4, non-B, non-T cells produce IL-3, but no detectable interleukin 2 or interferon γ. Non-B, non-T cells may be an important source of lymphokines in a variety of immune responses and may serve to amplify the effects of T cells of the T_{H2} type.**

Interactions between immunocompetent cells are largely mediated by the action of a set of polypeptide factors, designated lymphokines (LK), that control the activation, growth, and differentiation of cells of the immune system and of related cell populations (1). LK production by T lymphocytes is particularly important in regulating the functions of all cells that participate in immune responses. Recent evidence suggests that other hematopoietic lineage cells may also be important producers of "T-cell-derived" LKs. Many transformed murine mast cell lines express mRNA for and secrete one of these LK, interleukin 4 (IL-4) (2). Some lines also express mRNA for interleukin 3 (IL-3) and granulocyte/macrophage colony-stimulating factor.

The demonstration that the IL-3-dependent myeloid cell line FDC/1 produces IL-3 and IL-4 upon exposure to immune complexes indicated the potential importance of Fc receptors in stimulating LK production by hematopoietic cells other than T cells (3). We (4) and others (5) subsequently showed that long-term, IL-3-dependent mast cell lines produced IL-3, IL-4, interleukin 5 (IL-5), and interleukin 6 (IL-6), but not interleukin 2 (IL-2), interferon γ (IFN-γ) or lymphotoxin in response to calcium ionophores or to cross-linkage of their high-affinity Fcε receptors. Cross-linkage of Fcε receptors on mast cell lines has also been reported to lead to the release of other polypeptides, including interleukin 1 (IL-1) and several of the macrophage inflammatory proteins (6).

These results raised the possibility that normal mast cells and other nonlymphoid hematopoietic lineage cells might participate in immune responses by production of LKs. In this report, we show that a cell population lacking B- and T-cell markers, in the spleen of normal mice, produces IL-4 when stimulated by cross-linkage of receptors for Fc portions of IgE or IgG subclasses. IL-3 greatly enhances (for IgE) or is required (for IgG) for IL-4 production by non-B, non-T cells from naive mice. This production of IL-4 in response to Fc receptor cross-linkage could be an important mechanism in immunologic inflammation in allergic diseases, in infections with helminthic parasites, as well as in diseases marked by accumulation of immune complexes.

MATERIALS AND METHODS

Animals. Virus-free BALB/c female mice, 8–12 weeks of age, were obtained from the Division of Cancer Therapy Animal Program, National Cancer Institute.

Preparation of Non-B, Non-T Cells, B Cells, and T Cells. Erythrocyte-depleted spleen cells were incubated at 4°C for 1 hr with a mixture of purified (7) fluoresceinated (F1) antibodies containing anti-Thy-1.2 (30H12) (8), anti-CD3 (2C11) (9), anti-CD4 (Gk1.5) (10), anti-CD8 (2.43) (11), anti-B220 (6B2) (12), and anti-Ia^d (MKD6) (13) at 10 μg/ml to yield non-B, non-T cells. B cells were prepared by incubating the spleen cells with anti-T-cell reagents (anti-Thy-1.2, anti-CD3, anti-CD4, and anti-CD8); T cells were prepared by incubating the spleen cells with the anti-B-cell reagents (anti-B220 and anti-Ia^d). At the end of the incubation, the cells were washed twice in staining buffer and mixed for 1 hr at 4°C with a suspension of magnetic beads coated with sheep anti-F1 antibodies (Advanced Magnetics, Cambridge, MA) (12 ml per 10^8 stained cells). Positively staining cells were depleted by two 20-min cycles of exposure to a magnetic field. The remaining cells were washed twice in culture medium and examined for the removal of the desired cell populations (B, T, or both B and T cells) by cytometric analysis on a FACScan (Becton Dickinson). The non-B, non-T-cell population contained <10% stained cells.

Lymphokines and Cytokines. Synthetic IL-3 was a gift of Ian Clark-Lewis (Biomedical Research Center, Vancouver, Canada). One unit of IL-3 was defined as the amount of IL-3 that stimulated half-maximal [^{3}H]thymidine incorporation by FDC/1 cells. Mouse recombinant IL-4 was obtained from a baculovirus expression system, utilizing a vector into which the IL-4 gene had been inserted by C. Watson (Laboratory of Immunology, National Institute of Allergy and Infectious Diseases). IL-4 activity was measured using the CT.4S cell

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Abbreviations: IFN-γ, interferon γ; IL-1, -2, -3, -4, -5, -6, interleukin 1–6, respectively; LK, lymphokine(s); NK, natural killer; PB, plate bound; Fl, fluoresceinated; FcγRII, Fcγ receptor II.

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line (14), utilizing a standard that had initially been calibrated on the basis of 10 units/ml being equal to the amount of IL-4 required for half-maximal stimulation of [^{3}H]thymidine uptake by resting B cells stimulated with 5 μg of goat anti-IgM antibody per ml (15, 16).

Immunoglobulins. Purified mouse IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM were purchased from Southern Biotechnology Associates (Birmingham, AL). Monoclonal mouse IgE (HI-DNP-ε26) (17) was purified from ascites fluid by ammonium sulfate precipitation, DE52 ion-exchange chromatography, and gel filtration. Affinity-purified goat anti-mouse IgD antibody was prepared as described elsewhere (7).

Measurement of Lymphokine Production from Cells Stimulated by Plate-Bound (PB) Antibodies. Flat-bottom 96-well microtiter Immulon 2 plates (Dynatech Laboratories) were coated by incubation for 4 hr at room temperature with purified immunoglobulin (10 μg/ml in 50 μl of borate-buffered saline, pH 8.5). Wells were washed three times with 200 μl of Hanks' balanced salt solution. Cells were added to the antibody-coated wells (in triplicate) in 0.2 ml of culture medium. After 36 hr in culture, the plates were exposed to 1000 R (1 R = 0.258 mC/kg) in a γ Cell 40 Atomic Energy (Ottawa) irradiator. Lymphokine-dependent cells [CT.EV, an IL-2-dependent line (14); CT.4S, an IL-4-dependent line (14); FDC/1, an IL-3-dependent line (3, 18)] (5000 cells per well) were added to measure the secretion of individual lymphokines. Forty-eight hours later, 1 μCi of [^{3}H]thymidine (ICN; 1 Ci = 37 GBq) was added, and after 9 hr the cells were harvested. Relative SEs had a mean value of 15% or less. The amount of lymphokine was expressed as either the quantity of [^{3}H]thymidine incorporated into the DNA of the indicator cell line or as the equivalent of the amount of lymphokine required to support the same level of thymidine incorporation in the indicator cell line ("equivalent units"). INF-γ was assayed by its ability to inhibit plaque formation by vesicular stomatitis virus on L929/2G1 cells (19).

Assay for Cytotoxic Activity. Serial 2-fold dilutions of non-B, non-T cells were dispersed, in 0.1-ml volumes, into V-bottom wells of 96-well culture plates (Linbro or Flow Laboratories). Five thousand YAC-1 target cells, labeled with ^{51}Cr (150 μCi for 45 min), were added in a volume of 0.1 ml. Plates were centrifuged at 200 $\times$ *g* for 5 min to pellet cells. Cultures were incubated at 37°C for 4 hr and were then centrifuged (200 $\times$ *g*, 5 min). A sample (50 μl) of supernatant was removed and assayed for percent specific lysis (20).

RESULTS

Non-B, Non-T Cells Produce IL-4 in Response to PB Immunoglobulin. B and T lymphocytes were removed from spleen cell suspensions by magnetic bead separation as described. The resultant cells, designated splenic non-B, non-T cells, were cultured at 20,000 cells per well on culture plates that had been coated with murine IgE or murine IgG2a or had been left uncoated. IL-4 production was measured by irradiating the cells at 36 hr of culture and adding 5000 CT.4S cells, an indicator line selective for IL-4 (14). [^{3}H]Thymidine was added 48 hr later. Non-B, non-T cells from untreated mice produced IL-4 in response to PB-IgE but usually failed to secrete IL-4 in response to PB-IgG2a (Fig. 1). A monoclonal anti-IL-4 antibody (11B11) (21) (2 μg/ml) completely inhibited CT.4S [^{3}H]thymidine uptake in cultures stimulated with PB-IgE, indicating that IL-4 was the LK measured in this system (data not shown). Neither B- nor T-cell populations produced IL-4 in response to PB-IgE or PB-IgG2a.

IL-3 Enhances Production of IL-4 by Non-B, Non-T Cells in Response to PB Immunoglobulins. In studies of non-B, non-T cells from mice infected with *Nippostrongylus brasiliensis* or polyclonally activated by injection of goat anti-mouse IgD

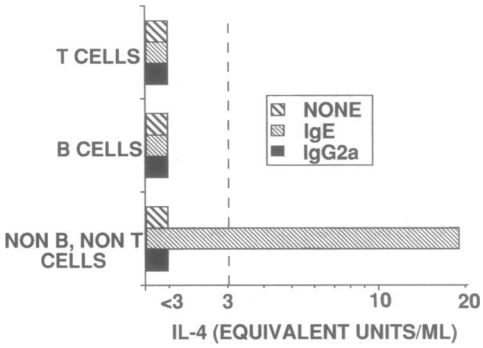

FIG. 1. Non-B, non-T cells produce IL-4 in response to PB immunoglobulin. Non-B, non-T cells (20,000 cells per well) obtained from untreated mice were incubated in 96-well plates that had been previously uncoated or coated with IgE (10 μg/ml) or IgG2a (10 μg/ml). After 36 hr, cultures were irradiated, and IL-4 production was measured by the addition of CT.4S cells (5000 cells per well). DNA synthesis by CT.4S cells was determined by incorporation of [^{3}H]thymidine 48 hr later. Results are expressed as IL-4 equivalent units. The assay can detect $\approx$3 units of IL-4 per ml; the dotted line indicates this cutoff of the sensitivity of the assay.

antibodies, we observed striking enhancement in IL-4 production in response to PB-IgE and observed that PB-IgG2a could also stimulate IL-4 production by non-B, non-T cells (34). This suggested that non-B, non-T cells might be induced to produce more IL-4 in response to PB-immunoglobulins. Because IL-3 is known to cause the growth and differentiation of many hematopoeitic lineage cells, non-B, non-T cells from normal mice were cultured with or without IL-3 on uncoated plates or on plates coated with IgE or IgG2a. Non-B, non-T cells, even at 2 $\times$ 10^5 cells per well, from untreated mice failed to produce IL-4 in response to PB-IgG2a; when cultured in the presence of IL-3, as few as 2000 non-B, non-T cells produced measurable IL-4 in response to PB-IgG2a (Fig. 2). PB-IgE does stimulate IL-4 production from normal non-B, non-T cells in the absence of IL-3. However, addition of IL-3 strikingly enhances IL-4 production.

IL-4 Is Produced in Response to PB-IgG1, IgG2a, and IgG2b as Well as to PB-IgE. PB immunoglobulins of several classes were tested, in the presence of IL-3, for their capacity to stimulate non-B, non-T cells to secrete IL-4. In this experiment, non-B, non-T cells were obtained from donors that had been treated with 800 μg of anti-IgD i.v. 5 days earlier to increase their production of IL-4 in response to PB immunoglobulins. IgG1, IgG2a, and IgG2b were of approximately

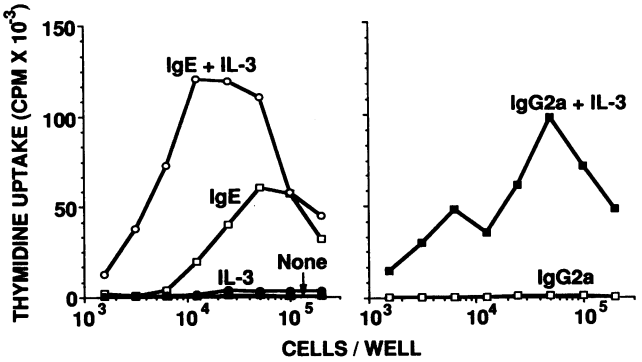

FIG. 2. IL-3 enhances IL-4 production in response to PB immunoglobulin. Non-B, non-T cells from untreated mice were incubated at various cell densities either without or with PB-IgE or PB-IgG2a in the presence or absence of IL-3 (50 units/ml). IL-4 production was measured by coculture with CT.4S cells and expressed in terms of [^{3}H]thymidine uptake by these cells.

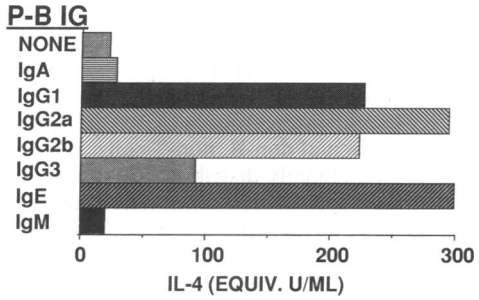

FIG. 3. IL-4 production by non-B, non-T cells in response to different classes of PB immunoglobulin. Non-B, non-T cells (20,000 cells per well) from mice that had been injected with anti-IgD 5 days earlier were cultured in the presence of IL-3 (50 units/ml) on plates that had been coated with each of the indicated mouse immunoglobulins, in the form of myeloma proteins at 10 μg/ml, as described. IL-4 production was measured as described in the legend to Fig. 1.

equal stimulatory capacity, whereas IgG3 stimulated smaller, but definite responses (Fig. 3). PB-IgM and PB-IgA failed to stimulate IL-4 production. PB-IgE was an excellent stimulant.

IL-4 production by non-B, non-T cells from anti-IgD-injected mice in response to PB-IgE is blocked by soluble IgE; 50% inhibition is obtained with <1 μg/ml (Fig. 4). Soluble IgE has only a marginal inhibitory effect on the response to PB-IgG2a, even at 1 mg/ml. The rat monoclonal antibody 2.4G2, specific for Fcγ receptor II (FcγRII) (22), is an efficient inhibitor of the response to PB-IgG2a but fails to significantly inhibit the response to PB-IgE. Soluble IgG2a is a poor inhibitor of the response to PB-IgG2a; in some experiments, 50% inhibition was observed at $\approx$30 μg/ml, but in other experiments, 50% inhibition was not achieved, even at higher concentrations. The distinctive pattern of inhibition by soluble IgE and 2.4G2 of the response to PB-IgE and to PB-IgG2a indicates that at least two separate receptors are involved in the induction of IL-4 production by non-B, non-T cells stimulated in the presence of IL-3. Based on the capacity of 2.4G2 to inhibit responses to PB-IgG2a, the receptor for that response appears to be FcγRII. Further, the capacity of soluble IgE to block stimulation by PB-IgE

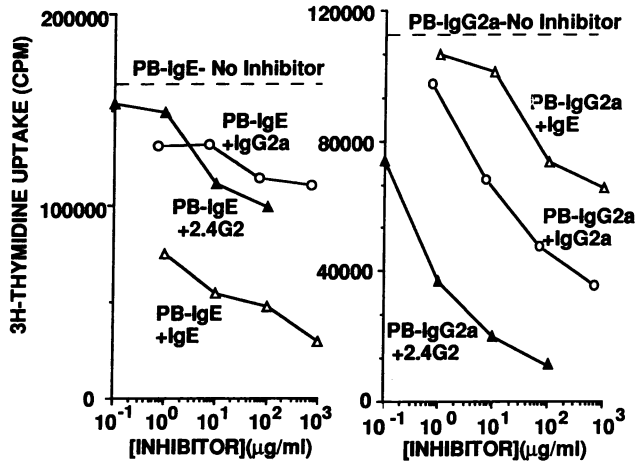

FIG. 4. Soluble immunoglobulin and anti-FcγRII receptor antibody inhibition of PB immunoglobulin stimulation of non-B, non-T cells. Non-B, non-T cells (20,000 cells per well) from mice that had been injected with anti-IgD 5 days earlier were incubated with soluble IgE, IgG2a, or 2.4G2 at the indicated concentrations or with nothing and then added to culture wells coated with IgE or IgG2a. IL-4 production was measured by the addition of CT.4S cells as described in the legend to Fig. 2.

Table 1. PB-IgE-stimulated lymphokine production by non-B, non-T cells, units/ml

	Normal donor		Anti-IgD-injected	
Lymphokine	− IL-3	+ IL-3	− IL-3	+ IL-3
IL-2	<0.15	<0.15	<0.15	<0.15
IL-3	0.8	ND	30	ND
IL-4	<3	200	350	350
IFN-γ	<1	<1	<1	<1

Non-B, non-T cells were prepared from spleens of untreated and anti-IgD-injected mice. Cells were cultured in wells (25,000 cells per well) coated with IgE (10 μg/ml) with or without IL-3 (50 units/ml). Supernatant fluids were harvested at 48 hr, and lymphokine assays were done as outlined. ND, not determined.

indicates a need for multivalent binding to signal IL-4 production.

Neither IL-2 nor IFN-γ Are Produced by Non-B, Non-T Cells Stimulated by PB Immunoglobulins. We tested supernatants of non-B, non-T cells stimulated with PB-IgE for IL-2, IL-3, IL-4, and IFN-γ activity (Table 1). Non-B, non-T cells from untreated mice produced no detectable IL-4 in the absence of IL-3 but produced substantial amounts in its presence. (Our failure to detect IL-4 in the supernatants of non-B, non-T cells from untreated mice stimulated with PB-IgE in the absence of IL-3, although we had detected IL-4 production by these cells using the CT.4S co-culture assay, appears due to the substantially greater sensitivity of the latter assay.) IL-3 was produced in small amounts by non-B, non-T cells from normal mice and in substantial amounts by non-B, non-T cells from anti-IgD-injected mice. We have not yet tested the effect of IL-3 upon IL-3 production in these cells because of the protocol used in these experiments. Neither IL-2 nor IFN-γ were detected in supernatants of non-B, non-T cells from normal mice or anti-IgD-injected mice stimulated with PB-IgE in the absence or presence of IL-3.

Characterization of Non-B, Non-T Cells That Produce IL-4 in Response to PB Immunoglobulins. To further characterize the splenic non-B, non-T cells that produce IL-4 in response to PB immunoglobulin, we prepared splenic non-B, non-T cells from untreated mice and from anti-IgD-injected mice and removed Mac1$^+$ cells by treatment with Fl-anti-Mac1 and depletion with anti-Fl conjugated magnetic beads. The Mac1-depleted populations and control non-B, non-T cells were equivalent in IL-4 production in response to PB-IgE and PB-IgG2a. We also subjected splenic non-B, non-T cells to density-gradient centrifugation in Percoll. The great majority of the IL-4-producing activity was found in the 50–60% Percoll band. The cells in this band were moderately large cells with basophilic cytoplasm, consistent with their being hematopoietic progenitor cells. Few mature polymorphonuclear leukocytes were observed in the band.

To test whether the non-B-, non-T-cell populations that produced IL-4 in response to PB immunoglobulins were natural killer (NK) cells, we depleted NK cell activity by treatment of mice with anti-asialo-GM1 antibodies (20). Splenic non-B, non-T cells were prepared from these mice 1 day later. These cells were devoid of the capacity to lyse the NK-sensitive target cell YAC-1, indicating that NK activity had been depleted by this measure. Nonetheless, they showed enhanced IL-4 production in response to PB immunoglobulins, indicating that NK cells were not the major contributors to IL-4 production by non-B, non-T cells (Fig. 5).

DISCUSSION

These studies indicate that IL-4, as well as IL-3, but not IL-2 and IFN-γ, are produced by a spleen cell population that lacks T-cell (Thy-1, CD3, CD4 and CD8) and B-cell (B220,

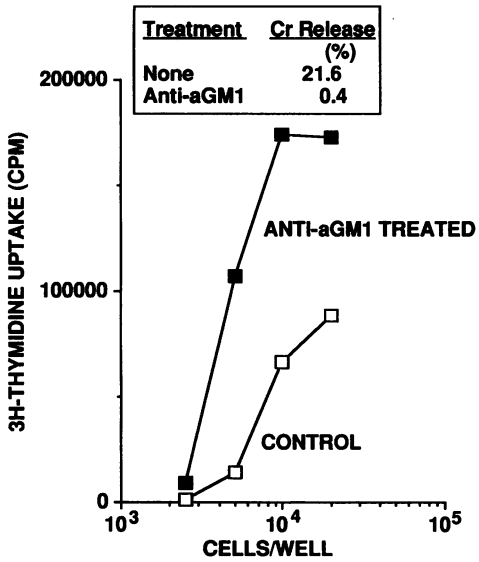

FIG. 5. Anti-asialo-GM1 treatment of mice does not deplete IL-4-producing non-B, non-T cells. Mice that had been treated with anti-IgD 4 days earlier were treated with anti-asialo-GM1 (100 μl of a rabbit antiserum). One day later, non-B, non-T cells were prepared from the spleens of these mice. Cytotoxic activity was tested with the NK-sensitive target cell YAC-1 as described. Results at an effector/target ratio of 10:1 are shown. IL-4 production by non-B, non-T cells (20,000 cells per well) from treated and control groups in response to PB-IgE and PG-IgG2a, in the presence of IL-3, was measured.

class II major histocompatibility complex) markers. LK production by such cells is stimulated by engagement of Fc receptors by PB immunoglobulins—most notably IgE and IgG1, IgG2a, and IgG2b. PB-IgM or IgA fail to stimulate these responses. Neither T cells nor B cells prepared from spleen produce IL-4 in response to PB immunoglobulins, making it highly unlikely that potentially contaminating T or B lymphocytes could be responsible for IL-4 production by non-B-, non-T-cell populations. NK cells have been reported to produce LKs, most notably IL-2 and IFN-γ (23, 24). However, we think it unlikely that NK cells are the major producers of IL-4 in response to PB immunoglobulins. (*i*) Many NK cells are Thy-1$^+$ (25). Such cells have been eliminated from our non-B, non-T-cell populations, and Thy-1$^+$ cells (i.e., "T cells") fail to produce IL-4 in response to Fc receptor cross-linkage. (*ii*) Treatment of mice with anti-asialo-GM1 completely depletes NK activity, as would be anticipated from the expression of asialo-GM1 on NK cell surfaces (20). The remaining non-B, non-T-cell population is enriched in IL-4-producing capacity. (*iii*) The non-B-, non-T-cell population that we have examined fails to produce IL-2 and IFN-γ, which are the LKs known to be made by NK cells (23, 24). These arguments do not rule out the possibility that NK cells can respond to PB immunoglobulins with IL-4 production. They do establish that other cells in the non-B-, non-T-cell population are excellent producers of IL-4.

The current work shows that the capacity of non-B, non-T cells to produce IL-4 depends upon, or is enhanced by, an IL-3-mediated event. Thus, normal non-B, non-T cells fail to produce IL-4 in response to PB-IgG2a and are relatively weak producers in response to PB-IgE. When cultured with IL-3, their capacity to produce IL-4 is markedly enhanced; as few as 2000 cells per well produce easily detectable IL-4 in response to either PB-IgG2a or PB-IgE under these conditions. By contrast, cells obtained from donors that had been injected with anti-IgD, or infected with *N. brasiliensis*, produce substantial amounts of IL-4 without need for addition of IL-3 (34). Both of these treatments lead to striking polyclonal increases in serum IgE concentration (26, 27). We interpret these findings to indicate that the capacity of non-B, non-T cells to secrete IL-4 (and presumably IL-3) is regulated by the *in vivo* action of T lymphocytes, presumably through the production of IL-3.

There are several interesting features of this proposed amplification function of non-B, non-T cells. Because non-B, non-T cells use nonclonally distributed receptors for antigen (i.e., "cytophilic" IgE or IgG antibodies) or for antigen–antibody complexes (i.e., the Fc receptors themselves), essentially all IL-3-stimulated cells potentially capable of producing LK could respond to immune complexes or, assuming that their Fc receptors have already bound the cognate antibody, to multivalent antigen. Thus, this population of non-B, non-T cells may represent many more LK-producing cells than do antigen-specific T cells and could markedly increase the number of LK-producing cells in a specific immune response. Furthermore, non-B, non-T cells are stimulated by a fluid-phase stimulant and thus could potentially produce IL-4 and related LKs for action at a distance, in contrast to T cells, which largely secrete these molecules for action in cognate interactions (28, 29).

Splenic non-B, non-T cells produce IL-4 but not IL-2 or IFN-γ. This is a pattern similar to that reported for the T_{H2} subset of cloned CD4$^+$ T cell lines (30). Non-B-, non-T-cell LK production may well act mainly to amplify T_{H2}-type immune responses.

The cells that produce IL-4 in this system have not been definitively identified, nor is it known whether the same cell responds to PB-IgE and PB-IgG. We have shown that removing Mac1$^+$ cells from the non-B-, non-T-cell population does not remove the IL-4-producing cells, indicating that cells with the phenotype Thy-1$^-$, CD3$^-$, CD4$^-$, CD8$^-$, B220$^-$, class II$^-$, Mac1$^-$ can produce IL-4 in response to Fc receptor crosslinkage. Among this population, it is the cells with intermediate density (i.e., those in the 50–60% Percoll band on density-gradient centrifugation) that produce IL-4. This population lacks mature granulocytes but does contain cells with the morphologic properties of hematopoietic progenitors.

The receptor mediating IL-4 production in response to PB-IgG2a appears to be FcγRII because the FcγRII-specific monoclonal antibody 2.4G2 blocks stimulation by PB-IgG2a but not by PB-IgE. The receptor involved in the response to PB-IgE has the characteristics of a high-affinity receptor since soluble IgE at 1 μg/ml can inhibit stimulation by PB-IgE. Furthermore, M. Plaut (personal communication) has shown that non-B, non-T cells can be sensitized with IgE, washed, incubated at room temperature for 2 hr, washed again, and still respond to anti-IgE by the production of IL-4. This result is consistent with our demonstration that a high-affinity Fcε receptor mediates release of LK from mast cell lines (4) and suggests that the relevant receptor is Fcε receptor I.

Because Fcε receptor I appears to be uniquely expressed on cells of mast cell and basophil lineages (31), such cells would seem responsible for IL-4 production in response to PB-IgE. However, mature mast cells are generally not found in mouse spleens (32). Indeed, by alcian blue staining, we have failed to observe cells with the characteristics of mature mast cells in the splenic non-B-, non-T-cell population of normal mice, although rare cells with an occasional alcian blue-positive granule were observed. Thus far, we have been unsuccessful in detecting IgE receptors on non-B, non-T cells by sensitization with IgE and staining with fluoresceinated anti-IgE, suggesting that the cells have relatively few IgE receptors. Although this argues strongly that the cells are not mature mast cells, the spleen is a source of mast cell precursors (32), and such cells are good candidates for the PB-IgE-stimulated IL-4-producing cells in the non-B-, non-T-cell population.

The demonstration that non-B, non-T cells from immunized animals are a rich source of IL-4 and related LKs and that cells from untreated mice can be stimulated to produce IL-4 by treatment with PB immunoglobulins and IL-3 provides strong support for the concept that physiologic production of "T-cell-derived" LKs is not limited to T cells but may, under the influence of T cells, be a property of cells of several hematopoietic lineages. Further, these results suggest that non-B, non-T cells may be potential sources of LKs in disorders marked by the presence of immune complexes as well as in allergies and nematode infections. Indeed, persistence of immune complexes in certain depots may lead to chronic production of LKs and resultant local exacerbation of cellular inflammatory responses. The recent report that tumor cell lines expressing IL-4 as a result of transfection fail to grow in nude mice and that IL-4-producing tumors inhibit the growth of other transplantable tumors injected at the same site suggests that IL-4 may have an important role in control of growth of malignant cells (33). Activation of non-B, non-T cells by immune complexes could provide a mechanism through which antibody may participate in anti-tumor responses by eliciting local IL-4 production.

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