Schistosoma mansoni Egg-induced Early IL-4 Production Is Dependent upon IL-5 and Eosinophils

By Elizabeth A. Sabin,* Manfred A. Kopf,* and Edward J. Pearce*

From the *Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401, and the † Basel Institute for Immunology, CH-4005, Basel, Switzerland

Summary

The initial immune response to Schistosoma mansoni eggs presumably results in IL-4 production, as schistosome eggs are strong Th2-inducing antigens and the differentiation of antigen-specific Th2 cells is largely dependent on the presence of IL-4 during priming of naive Th cells. Consistent with this concept, intraperitoneal injection of mice with schistosome eggs results in an upregulation of IL-4 production by peritoneal exudate cells (PECs) within 12 h. Egg-induced IL-4 is rapidly bound by its receptor, suggesting that this cytokine is utilized by a cell type present at the site of antigen deposition or is complexed to soluble receptor. The peak of early IL-4 production is accompanied by a local eosinophilia and the apparent disappearance of mast cells. Studies utilizing either IL-4, IL-5, or mast cell-deficient mice indicate that the eosinophilia is dependent on mast cells and IL-5 and independent of IL-4. Strikingly, egg-induced IL-4 production is absent in animals lacking the early peritoneal eosinophilia. Immunocytochemical analysis of PEC following egg injection indicates that the eosinophils themselves make IL-4. These data strongly suggest that egg-induced IL-5 plays an essential role in recruiting eosinophils to the site of antigen deposition and that it is these eosinophils that then directly produce early IL-4.

Blood and tissue eosinophilia are prominent features of immune responses generated during helminth infection, asthma, and allergies. In murine model systems of parasitic infections and airway hyperreactivity (asthma), the in vivo abrogation of IL-5 results in an ablation of the characteristic eosinophil response, demonstrating the central role played by this cytokine in eosinophil mobilization and maturation (1–5). IL-5 is secreted primarily by Th2 cells but other cell types (mast cells, other FcgR+ cells, and eosinophils themselves) prominent during type 2 immune responses may also contribute to its production (6, 7). Whereas IL-5 is central to eosinophilia, the function of the eosinophils themselves during the course of parasitic infections or as mediators of asthmatic airway hyperreactivity remains controversial (1–5, 8, 9). The majority of studies that have investigated the role of eosinophils have focused on the different arm of the immune response; eosinophil mobilization from the bone marrow and migration into tissues has largely been thought of as an antigen-specific Th2 cell–dependent event (10–12). However, recent studies of eosinophils indicate that these cells may play more of an afferent role than previously thought. Eosinophils can express MHC class II and present Ag to Th cells (13, 14); moreover, once activated they also secrete the T cell growth and differentiation factor IL-4 (15, 16).

IL-4, the prototypic type 2 cytokine, plays a crucial role in promoting the differentiation of naive precursor Th (pTh)1 cells into Th2 cells (17–20). To assert this effect, IL-4 must be available to pTh cells shortly following primary exposure to antigen. Because Th2 cells specific for a given antigen presumably do not exist during priming, other cell types have been proposed to contribute the essential early IL-4 (21–23). Most recently, NK+, CD4+ T cells were shown to play such a role during the induction of a Th2 response by anti-IgD (24). However, in our studies using Schistosoma mansoni eggs as a potent Th2 response inducer, early IL-4 production is T cell independent (25). While performing studies designed to identify the cellular source of the egg-induced early IL-4, we have found that eggs profoundly stimulate the IL-4 independent production of early IL-5 with an accompanying IL-5 dependent local eosinophilia. Moreover, the egg-induced IL-5 dependent eosinophilia is essential to allow production of the early IL-4. The IL-4 secreted subsequent to the peritoneal eosinophilia is rapidly bound by IL-4Rα, indicating that this cytokine is...
being utilized, perhaps by egg-antigen-specific pTh cells. These studies suggest a novel role for eosinophils in the inductive phase of type 2 immune response development and may help to explain previous studies, which have proposed conflicting roles for eosinophils in downstream type 2 responses (4, 5).

Materials and Methods

Parasites, Antigens, and Experimental Inoculations. *S. mansoni* (NMRI strain) eggs were recovered from the livers of infected mice as previously described (26, 27) and resuspended at 10^6/ml in sterile, low endotoxin PBS (Sigma, St. Louis, MO). The eggs were determined to be free of endotoxin using a timed-gel endotoxin kit (Sigma) and stored at -70°C until use. Wild type (WT) C57BL/6 mice were from Taconic Farms (Germantown, NY). Athymic (nude) C57BL/6 and WBB6F1-W/Wv plus their heterozygote littersmates were from the Jackson Laboratory (Bar Harbor, ME). Homozygous C57BL/6 IL-4/-/- mice were derived from 129Sv × C57BL/6 IL-4/-/- mice (28) by eight backcrosses to C57BL/6 WT mice and interbreeding of heterozygote offspring of the final backcross. C57BL/6 IL-5/-/- mice were the offspring of breeding colonies established from previously described animals (29). Female 6-10-week-old mice were injected intraperitoneally with 10,000 *S. mansoni* eggs in 10 µl PBS or with an equivalent volume of PBS alone.

Cell Preparation and Identification. Mice were euthanized with CO2. PECs were collected by lavage with 10 ml of ice-cold HBSS containing 1% FCS and 10 mM Hepes (all from Sigma). Viable PECs were enumerated using Trypan blue exclusion and resuspended at 5 × 10^6/ml in complete tissue culture medium (CTCM) containing DMEM, 10% FCS, 5 × 10^-5 M 2-ME (all from Sigma), plus 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 30 mM Hepes (all from Gibco BRL, Gaithersburg, MD). Cytospins of 10^6 PEC per slide were stained with Wright's stain and cell populations enumerated by light microscopy.

Ex Vivo Cytokine Production. PECs collected from mice injected 12 h previously were aliquoted into 96-well flat-bottomed plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ; 1.25 × 10^6 cells in 250 µl CTCM per well) and cultured at 37°C/5% CO2 in the presence or absence of a blocking mAb directed against the IL-4R (M1 at 2.5 µg/ml; a gift from Immunex, Seattle, WA) and with or without ionomycin (4 µg/ml; Calbiochem-Novabiochem, San Diego, CA). Supernatants from 24 h cultures were collected and cytokine levels quantitated using highly specific two-site ELISAs described in detail elsewhere (25, 30). IL-4 levels are expressed in units as defined by Genzyme Diagnostics (Cambridge, MA). Statistical analyses were performed using Student's t test; P values <0.05 are considered significant.

In Vivo Neutralization of IL-5. C57BL/6 mice were intraperitoneally injected 24 h before and again immediately before *S. mansoni* egg injection with 1 mg of neutralizing anti-IL-5 mAb (TRFK5; American Type Culture Collection, Rockville, MD; mAb purified on protein G) or with an equivalent amount of normal rat γ-globulin (Accurate Chemical and Scientific Corp., Westbury, NY). 12 h after intraperitoneal injection, PECs were collected, stimulated in vitro, and cytokine levels in cell culture supernatants analyzed as described above.

Immunocytochemistry. Cytospin preparations of PECs collected from WT or IL-4/-/- mice 12 h following intraperitoneal injection were fixed, permeabilized, and stained with either a biotinylated mAb anti-IL-4 (protein G-purified 11B11 at 4 µg/ml) or a biotinylated isotype control mAb (rat IgG1 at 4 µg/ml; Pharmingen, San Diego, CA), following a previously described protocol (31). Nonspecific Ab binding via FcyR was blocked by incubation with mAb anti-CD16/CD32 (0.5 µg/ml; Pharmingen) before incubation with biotinylated mAbs. Detection was with alkaline phosphatase-conjugated streptavidin (1:10,000; Jackson Immunoresearch Laboratories, West Grove, PA) followed by BCIP/NBT (5-bromo-4-chloro-3-indolyl-1-phosphate and nitro blue tetrazolium; Promega, Madison, WI) according to the suggested protocol of the company.

Determination of Cytokine Levels in Serum and Peritoneal Lavage Media. 12 h after intraperitoneal injection, mice were bled from the tail vein and serum stored at -20°C for subsequent quantitation of cytokine levels by ELISA. Mice were then euthanized and PECs collected as described above. Cell-free lavage media (9–10 ml/mouse) were concentrated to final volumes of 0.75–1 ml with Centriprep-10 concentrators (Amicon, Beverly, MA) and stored at -20°C for subsequent quantitation of cytokine levels.

Results and Discussion

IL-4 Secreted by Peritoneal Exudate Cells Is Rapidly Bound by Its Receptor. Previously, we described the induction of thymic-independent IL-4 gene transcription in PECs within 2–12 h of intraperitoneal injection of *S. mansoni* eggs (25).
Because cytokine production is primarily regulated at the mRNA level (32, 33), we were surprised when we were unable to demonstrate IL-4 protein accumulation in PEC culture supernatants using an ELISA. It occurred to us that IL-4 secreted into culture supernatants may be rapidly utilized, thus precluding its measurement by conventional ELISA. To address this issue, PECs collected 12 h after intraperitoneal injection were cultured in the presence or absence of a mAb directed against the IL-4R (αIL-4R; 34). Only when αIL-4R was included in the culture medium were we able to measure constitutive IL-4 secretion by PECs collected from egg-injected mice (2.29 ± 0.62 U/ml; Fig. 1 A). PECs from PBS-injected mice failed to yield measurable levels of IL-4 spontaneously, regardless of whether αIL-4R was present (Fig. 1 A).

Although we have consistently observed constitutive production of IL-4 by PECs 12 h after intraperitoneal egg injection, the absolute quantities of cytokine secreted into culture supernatants are always low. To increase our ability to measure IL-4, we stimulated PECs in vitro with the calcium ionophore, ionomycin (since IL-4 production by T cells [35] and FcεR+ cells [36, 37] can be promoted by increased cytoplasmic Ca2+). The amount of IL-4 secreted by PECs from egg-injected animals following in vitro ionomycin stimulation (in the presence of αIL-4R) increased 150 to 250-fold over that produced spontaneously (434.13 ± 101.12 versus 2.29 ± 0.62 U/ml; compare Fig. 1, B with A). Under these same conditions, IL-4 can be detected in the culture supernatants of PECs from PBS-injected mice, albeit at significantly lower levels than from egg-injected mice (55.09 ± 7.22 versus 434.13 ± 101.12, P <0.005; Fig. 1 B). Moreover, ionomycin stimulation promotes sufficient IL-4 secretion to allow IL-4 to accumulate to detectable levels even in the absence of αIL-4R (Fig. 1 B).

The increase in measurable levels of egg-stimulated IL-4 production by PECs in the presence of αIL-4R strongly suggests that secreted IL-4 is rapidly bound by its receptor. Receptors specific for IL-4 can be found either in the extracellular milieu in a soluble form or on the surface of such IL-4 responsive cells as T cells, B cells and mast cells (38). If the IL-4 produced early following intraperitoneal injection of S. mansoni eggs is important for the induction of Th2 cell differentiation, then it might be expected that cell surface IL-4R, on pTh cells would rapidly bind the IL-4 secreted into the peritoneal fluid during the initial exposure to egg antigens. In addition, soluble IL-4R may serve as an IL-4 carrier protein (39, 40), extending the half-life of this cytokine and allowing IL-4 produced by PECs at the site of antigen deposition to circulate easily to the draining thoracic lymph node, where both antigen presentation and Th2 cell differentiation may occur. We are currently investigating whether soluble IL-4R concentrations increase in the peritoneal fluid at times coincident with peak egg-induced IL-4 production.

Egg-induced Early IL-4 Is Absent in Mast Cell-Deficient Mice. Intraperitoneal injection of C57BL/6 mice with S. mansoni eggs not only results in early IL-4 gene transcription and protein production but also, over a similar time frame, in a decrease in the number of recoverable peritoneal mast cells and an increase in eosinophil numbers (25). Similar alterations in peritoneal eosinophil and mast cell numbers occur in egg-injected nude and CD4+ cell-depleted animals (25); PECs from T cell-depleted mice also make early IL-4 (for nude versus WT mice; 12 h PEC from egg-injected animals constitutively made 0.89 ± 0.13 versus 0.89 ± 0.11 U/ml IL-4). Together, these data strongly suggest that the egg-induced early changes in peritoneal cell composition and IL-4 production are T cell independent. Having excluded thymus-dependent T cells as the source of early IL-4, we asked which other cell types in the PEC population might be responsible for the production of this cytokine. Because murine mast cell lines can secrete IL-4 upon activation with calcium ionophores or other appropriate stimuli (36), and because the apparent decrease in recoverable peritoneal mast cells after egg inject-

---

**Figure 2.** Egg-induced early IL-4 production and eosinophil infiltrate are blocked in mast cell-deficient mice. WBB6F1-W/Wv (W/Wv) or heterozygote intermediates (W/+), or mast cell-indeficient W/Wv mice (W/Wv) were injected intraperitoneally with S. mansoni eggs. 12 h later, PECs from three mice per group were combined and cultured in duplicate in the presence of anti-IL-4R, alone (BKG), or plus ionomycin (IONO) for an additional 24 h. IL-4 levels in culture supernatants from PEC were measured twice by ELISA (A, note different scales on x axes). Differential counts were performed on Wright's stained cytospin preparations of PECs before culturing. The eosinophils are the mean ± SE from two individual experiments; eosinophil differentials are the mean ± SE from a total of six distinct fields of 100 cells from the same experiments described in (A).
jected with eggs, and 12 h later the ability of their PECs to most striking difference between PEC composition in egg-
levels of IL-4 when recovered at 12 h after intraperitoneal
mast cells) transferred from naive WT mice to the perito-
lowing stimulation in vitro with ionomycln (74.97 +
mice; PECs from W/+ mice constitutively secreted a small
detect spontaneous IL-4 production by PECs from W/Wv
staining with anti-IL-4 are indicated with a broad arrowhead (note staining in center of circular nuclei). Unstained eosinophils in the left panel are shown with narrow arrows, unstained eosinophils are also evident in center and right panels (not arrowed). A positively stained eosinophil with granules to side of nucleus is shown in inset of center panel; a control cell of similar appearance is shown in the middle of the left panel. Original magnification, X815.

tion could be due to their activation and subsequent de-
granulation or adherence to the peritoneum, we addressed
the role of mast cells in early egg-induced IL-4 production. Mice carrying a mutation in the gene encoding c-kit and thus congenitally deficient of mast cells (W/Wv) or their heterozygote mast cell intact littermates (W/+ ) were injected with eggs, and 12 h later the ability of their PECs to secrete IL-4 was analyzed (Fig. 2 A). We were unable to detect spontaneous IL-4 production by PECs from W/Wv mice; PECs from W/+ mice constitutively secreted a small but measurable amount of IL-4 (0.21 ± 0.06 U/ml, Fig. 2 A). IL-4 production by PECs from egg-injected W/Wv mice was also significantly less than that of W/+ mice following stimulation in vitro with ionomycin (74.97 ± 27.66 versus 346.98 ± 102.26 U/ml, P <0.05; Fig. 2 A).

The most straightforward explanation for the defect in early IL-4 production observed in the W/Wv mice is that directly make early IL-4. To address this issue experimentally, two types of eosinophil-deficient mice were utilized: IL-5−/− mice and C57BL/6 mice injected with a neutralizing anti-IL-5 mAb (TRFK5).

IL-5−/− mice maintain a near normal basal number of blood and bone marrow eosinophils while being unable to mount a blood or tissue eosinophilia (29). 12 h after intraperitoneal egg injection in these mice, IL-4 production by PECs is significantly less than that seen in egg-injected WT animals, regardless of the in vitro stimulus (P <0.005; Fig. 3 A). As with the W/Wv mice, we were unable to detect spontaneous secretion of IL-4 by PECs from IL-5−/− mice, while PECs from WT animals did secrete this cytokine without further in vitro stimulation (1.44 ± 0.32 U/ml; Fig. 3 A). As expected, IL-5−/− mice fail to mount a peritoneal eosinophilia in response to egg injection (Fig. 3 C).

Figure 3. IL-5 and eosinophils are necessary for early egg-
induced IL-4 production. (A and B) WT, IL-5−/− or mAb anti-
IL-5 (TRFK5) treated mice were injected intraperitoneally with S. mansoni eggs. 12 h later, PECs from three mice per group were combined and cultured in duplicate in the presence of anti-IL-4R either alone (BKG) or with ionomycin (IONO) for an additional 24 h. IL-4 levels in culture supernatants from PECs collected from WT or IL-5−/− mice (A) or from C57BL/6 mice treated with normal rat γ-globulin (NRAT) or with TRFK5 (B) were measured twice by ELISA. Note different scales on x axes in A and B. IL-4 levels are the mean ± SE of all measurements from three individual experiments (C) Differential counts were performed on Wright’s stained cytopsin preparations of PECs before culturing and per-
percentage of eosinophils are shown. Data are the mean ± SE from 9 distinct fields of 100 cells from the experiments described in A and B. (D) Cytosn preparations of PECs from egg-rejected WT mice were fixed, permeabilized, and immunostained with mAb anti-IL-4 (center and right) and an isotype control mAb (left) and counterstained with Wright’s Eosinophil staining with anti-IL-4 are indicated with a broad arrowhead (note staining in center of circular nuclei). Unstained eosinophils in the left panel are shown with narrow arrows, unstained eosinophils are also evident in center and right panels (not arrowed). A positively stained eosinophil with granules to side of nucleus is shown in inset of center panel; a control cell of similar appearance is shown in the middle of the left panel. Original magnification, X815.
Because a lack of IL-5 during development may have as yet undetermined effects on the IL-5−/− animals which might then influence their ability to respond to S. mansoni egg antigens, we also neutralized in vivo IL-5 by intraperitoneally injecting WT C57BL/6 mice with the mAb TRFK5 immediately prior to schistosome egg injection. This resulted in a significant decrease in the peritoneal eosinophil infiltrate compared with that seen in controls injected with normal rat γ-globulin and eggs (P < 0.001; Fig. 3 C). Concomitantly, PECs collected from egg-injected anti-IL-5-treated animals also exhibited a decreased ability to secrete IL-4 when compared with control PECs, again regardless as to the in vitro stimulus (Fig. 3 B). Moreover, we were unable to measure spontaneous egg-induced IL-4 production by PECs from TRFK5-treated animals, while constitutive IL-4 produced by control PECs was easily measurable (2.23 ± 0.99 U/ml; Fig. 3 B).

Taken together, the above data suggest that the egg-induced early IL-4 production is dependent on IL-5. A plausible explanation for this is that IL-5 made at the site of egg deposition is acting on bloodstream eosinophils to promote their ability to migrate into the peritoneal cavity (41, 42), and that it is these eosinophils which make the early IL-4. IL-5 could additionally be acting as an eosinophil chemoattractant, but data supporting such a role for this cytokine are conflicting (41, 43, 44), raising the possibility that another molecule, for example eotaxin (45, 46), C5a (47), or IL-4 (48, 49), is fulfilling this function.

Support for the hypothesis that egg injection stimulates IL-4 production specifically by eosinophils is provided by immunocytochemical analysis of permeabilized PECs, since mAb anti-IL-4 bound only to a subpopulation (12%) of cells with the nuclear morphology of eosinophils (Fig. 3 D, center and right). An isotype control Ab failed to bind to cells with this nuclear morphology (Fig. 3 D, left). Furthermore, mAb anti-IL-4 did not bind to PEC from egg-injected IL-4−/− mice (data not shown). That only a small percentage of PEC was positive for IL-4 (<0.05%) is consistent with previous ELISPOT results (25).

Egg-induced Peritoneal Eosinophilia Is Dependent on IL-5 and Independent of IL-4. If IL-5 is acting directly as an eosinophil chemoattractant to promote peritoneal eosinophilia, then the data presented above would predict that PECs from egg-injected mice should secrete more IL-5 than those from control PBS-injected animals. Surprisingly, we were unable to measure any differences in the ability of PEC from egg- versus PBS-injected C57BL/6 animals to make IL-5; both secreted IL-5 in response to ionomycin (data not shown). However, we were able to measure significantly elevated levels of IL-5 in both serum and lavage fluid from egg-injected compared with PBS-injected mice (lavage fluid values, 24.56 ± 11.11 versus 0 ± 0 pg/ml; P < 0.05; serum values, 297.23 ± 95.52 versus 29.35 ± 23.73 pg/ml; P < 0.05; Fig. 4, B and C). One interpretation of these data is that the cell type responsible for the egg-induced production of IL-5 is adherent to the peritoneum and therefore is not recoverable by lavage. That mast cells might constitute this cell type is suggested by the finding that following egg injection, serum and lavage fluid IL-5 levels are significantly lower in W/Wv mice compared with their mast cell-intact littermates (serum values, 105.45 ± 55.68 versus 492.14 ± 22.51 pg/ml; P < 0.001; lavage fluid values, 32.99 ± 20.91 versus 93.15 ± 9.69 pg/ml, P < 0.05).

IL-4 is also ascribed eosinophil attractant properties due to its ability to upregulate endothelial cell expression of VCAM-1, which in turn increases eosinophil binding to endothelium through cell surface expression of VLA-4 (48, 49). Thus, we were interested in the interplay of IL-5, IL-4, and eosinophils at early time points following intraperitoneal schistosome egg injection, as it was possible that...
the peritoneal eosinophilia induced by egg injection was affected by egg-induced early IL-4 production and not vice versa. Consequently, WT and IL-4−/− mice were intraperitoneally injected with S. mansoni eggs or PBS, and 12 h later the extent of peritoneal eosinophilia was determined from Wright's stained cytospin preparations of PECs. Compared with PBS-injected mice, egg-injected WT and IL-4−/− mice exhibited significantly increased numbers of eosinophils in their PEC populations (P < 0.05 for both types of mice), although the absolute magnitude of the eosinophilia was significantly lower in the IL-4−/− mice than in the WT animals (P < 0.01; Fig. 4 A). Consistent with a role for IL-5 in eosinophil recruitment, levels of this cytokine not only increased in the lavage media and sera of WT animals following egg injection, but were also significantly elevated in both the peritoneal lavage media and sera of egg-injected versus PBS-injected IL-4−/− animals (lavage media, 11.14 ± 5.17 versus 0 ± 0 pg/ml, P < 0.05; serum, 150.13 ± 53.56 versus 2.81 ± 1.64 pg/ml; P < 0.05; Fig. 4, B and C); the apparent differences in IL-5 levels between WT and IL-4 mice were not significant (lavage media, P = 0.31; serum, P = 0.22). These results demonstrate that egg-induced IL-5 production and eosinophils can occur in the absence of IL-4, but suggest that IL-4 may promote eosinophil infiltration through a mechanism unrelated to the upregulation of IL-5 per se. The data do not rule out the possibility that once made, IL-4 plays a role in a positive feedback loop to promote further early production of IL-5.

We describe here studies that indicate that S. mansoni eggs, potent Th2-inducing antigens, promote not only IL-4 secretion but also IL-3 production and tissue eosinophilia within 12 h of intraperitoneal injection into naive mice; previous studies from our laboratory indicate that this eosinophilia is T cell independent. The production of IL-4 by egg-stimulated PECs does not occur if IL-5 is unavailable or if peritoneal eosinophilia does not develop. Moreover, egg-induced IL-4 secreted by PECs is rapidly bound by its receptor, indicating that, at the time of antigen priming, a cell type within the PECs is utilizing IL-4 or is secreting IL-5. We are currently underway to investigate the relative importance of the IL-5/eosinophil-dependent mechanism of egg-induced IL-4 production.
References

1. Coffman, R.L., B.W.P. Seymour, S. Hudak, J. Jackson, and D. Reumick. 1989. Antibody to interleukin 5 inhibits helminth-induced eosinophilia in mouse. Science (Wash. DC). 245:308-310.
2. Sher, A., R.L. Coffman, S. Henry, P. Scott, and A.W. Cheever. 1990. Interleukin 5 (IL-5) is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with Schistosoma mansoni. Proc. Natl. Acad. Sci. USA. 87: 61-65.
3. Mauser, P.J., A. Pitman, A. Witt, X. Fernandez, J. Zurcher, T. Kung, H. Jones, A.S. Wattnick, R.W. Egan, W. Kreutner, and G.K. Adams. 1993. Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. Am. Rev. Respir. Dis. 148:1623-1627.
4. Corry, D.B., H.G. Folkesson, M.L. Warnock, D.J. Erie, and G.K. Adams. 1993. Inhibitory effect of the TRFK-5 receptor system: implications in the immune system and inflammation. Adv. Immunol. 57:145-190.
5. Foster, P.S., S.P. Hogon, A.J. Ramsay, K.I. Matthaei, and I.G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J. Exp. Med. 183:195-201.
6. Takatsu, K., S. Takaki, and Y. Hatoshi. 1994. Interleukin-5 and its receptor system: implications in the immune system and inflammation. Adv. Immunol. 57:145-190.
7. Desreumaux, P.A., J.F. Janin, J.F. Colombel, L. Prin, J. Phumas, D. Fmile, G. Torpier, A. Capron, and M. Capron. 1992. Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. J. Exp. Med. 175:293-296.
8. Bosquet, J., J.P. Chanez, J.Y. Barone, N. Chavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, and F.-B. Michel. 1990. Eosinophilic inflammation with tissue eosinophilia. N. Engl. J. Med. 323:1033-1039.
9. Ishida, K., R.J. Thomson, L.L. Beattei, B. Wiggs, and R.R. Schellenberg. 1990. Inhibition of antigen-induced airway hyperresponsiveness, but not acute hypoxia nor airway eosinophilia, by an antagonist of platelet-activating factor. J. Immunol. 144:3907-3911.
10. Durham, S.R., S. Ying, V.A. Varney, M.R. Jacobson, R.M. Suddenick, I.S. Mackay, A.B. Kay, and Q.A. Hamid. 1992. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophils. J. Immunol. 148:2390-2394.
11. Urban, J.F., K.B. Madden, A. Svetic, A. Cheever, P.P. Trotta, W.C. Gause, I.M. Katona, and F.D. Finkelman. 1992. The importance of Th2 cytokines in protective immunity to nematodes. Immunol. Rev. 127:205-220.
12. Garbi, C.G., A. Fakou, T.T. Kung, D. Stello, K.J. Peninille, A.J. Beavis, S.R. Smith, R.W. Egan, and S.P. Umland. 1995. T cells are necessary for Th2 cytokine production and eosinophil accumulation in airways of antigen-challenged allergic mice. Clin. Immunol. Immunopathol. 75:75-83.
13. Del Pozo, V., B. De Andres, E. Martin, B. Cardaba, J.C. Fernandez, S. Gallardo, P. Tramón, F. Leyva-Cobian, P. Palomo, and C. Lafoz. 1992. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. Eur. J. Immunol. 22:1919-1925.
14. Weller, P.F., T.H. Rand, T. Barrett, A. Elovic, D.T.W. Wong, and R.W. Finberg. 1993.Accessory cell function of human eosinophils. J. Immunol. 150:2554-2562.
15. Moorebel, R., S. Ying, J. Barkans, T.M. Newman, P. Kimmigt, M. WakeJn, L. Taborda-Barata, Q. Meng, C.J. Corrigan, S.R. Durham, and A.B. Kay. 1995. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. J. Immunol. 155:4939-4947.
16. Nonaka, M., R. Nonaka, K. Woolley, E. Adelroth, K. Miura, Y. Ohkawara, M. Glibetic, K. Nakano, P. O'Byrne, J. Dolovich, and M. Jordan. 1995. Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. J. Immunol. 155:3234-3244.
17. Le Gros, G.S., Z. Ben-Sasson, R. Seder, F.D. Finkelman, and W.E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. J. Exp. Med. 172:921-929.
18. Hsieh, C.S., A.B. Heinberger, J.S. Gold, A. O'Garra, and K.M. Murphy. 1992. Differential regulation of T helper phenotype development by interleukin 4 and 10 in an 0+ T-cell receptor transgenic system. Proc. Natl. Acad. Sci. USA. 89: 6065-6069.
19. Swain, S.L., A.D. Weinburg, M. English, and G. Huston. 1990. IL-4 and IFN-γ direct the development of distinct subsets of helper T cells. J. Immunol. 135:3796-3801.
20. Seder, R.A., W.E. Paul, M.M. Davis, and B.F. de St. Groth. 1992. The presence of interleukin 4 during in vivo priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. J. Exp. Med. 176:1091-1098.
21. Gollob, K.J., and R.L. Coffman. 1994. A minority subpopulation of CD4+ T cells directs the development of naive CD4+ T cells into IL-4 secreting cells. J. Immunol. 152:5180-5188.
22. Yoshimoto, T.O., and W.E. Paul. 1994. CD4+ NK1.1+ T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. J. Exp. Med. 179:1285-1295.
23. Ferrick, D.A., M.D. Schrenzel, T. Mulvania, B. Hseh, W.G. Fehn, and H. Lepper. 1995. Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by γδ T cells in vivo. Nature (Lond.) 373:255-257.
24. Yoshimoto, T., A. Bendelac, C. Watson, J. Hu-Li, and W.E. Paul. 1995. Role of NK1.1+ T cells in a Th2 response and in immunoglobulin E production. Science (Wash. DC). 270: 1845-1847.
25. Sabin, E.A., and E.J. Pearce. 1995. Early IL-4 production by non-CD4+ cells at the site of antigen deposition predicts the development of a T helper 2 cell response to Schistosoma mansoni eggs. J. Immunol. 155:4844-4853.
26. Boros, D.L., and K.S. Warren. 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced by a soluble factor isolated from Schistosoma mansoni eggs. J. Exp. Med. 132:488-494.
27. Boctrur, F.N., T.E. Nash, and A.W. Cheever. 1979. Isolation of a polysaccharide antigen from Schistosoma mansoni eggs. J. Immunol. 122:39-45.
28. Kopf, M., G. Le Gros, M. Bachmann, M.C. Lammers, H. Bluthmann, and G. Kohler. 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature (Lond.) 362:245-248.
29. Kopf, M., F. Brombacher, P.D. Hodgkin, A.J. Ramsay, E.A. Milbourne, W.J. Dai, K.S. Ovvington, C.A. Behm, G. Kohler, I.G. Young, and K.I. Matheai. 1996. IL-5 deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity.* 4:15-24.

30. Vella, A.T., and E.J. Pearce. 1994. *Schistosoma mansoni* egg-prime Th1 and Th2 cells: failure to downregulate IFN-γ production following in vitro culture. *Scand. J. Immunol.* 39: 12-18.

31. Sander, B., J. Andersson, and U. Andersson. 1991. Assessment of cytokines by immunofluorescence and the paraform-aldehyde–saponin procedure. *Immunol. Rev.* 119:65-94.

32. Brons, K.A., B. Beverly, S.-M. Kang, M. Lenardo, and R.H. Schwartz. 1991. Transcriptional regulation of cytokine genes in nontransformed T cells. *J. Immunol.* 147:3601-3609.

33. Murphy, K.M., T.L. Murphy, J.S. Gold, and S.J. Szabo. 1993. Current understanding of IL-4 gene regulation in T cells. *Res. Immunol.* 144:575-578.

34. Beckmann, M.P., K.A. Schooley, B. Gallus, T. VandenBos, D. Friend, A.R. Alpert, R. Raunio, K.S. Prickett, P.E. Parker, and L.S. Park. 1990. Monoclonal antibodies block murine IL-4-receptor function. *J. Immunol.* 144:4212-4217.

35. Arai, N., Y. Naito, M. Watanabe, E.S. Masuda, Y. Yamaguchi-Iwai, A. Tsuobu, T. Heke, I. Matsuda, K. Yokota, N. Koyano-Nakagawa, J.L. Hyun, M. Muramatsu, T. Yokota, and K.-I. Arai. 1992. Activation of lymphokine genes in T cells: role of cis-acting DNA elements that respond to T cell activation signals. *Pharmacol. Ther.* 55:303-318.

36. 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 FceRI or to calcium ionophores. *Nature (Lond.)* 339:64-67.

37. Seder, R.A., M. Plaut, S. Barbler, J. Urban, F.D. Finkelman, and W.E. Paul. 1991. Purified FceRI bone marrow and splenic non-B, non-T cells are highly enriched in the capacity to produce IL-4 in response to immobilized IgE. *IgG2a, or ionomycin. J. Immunol.* 147:903-909.

38. Mosley, B., M.P. Beckmann, C.J. March, R.L. Izdebera, S.D. Gimpel, T. VandenBos, D. Friend, A. Alpert, D. Anderson, J. Jackson et al. 1989. The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. *Cell.* 59:335-348.

39. Sato, T.A., M.B. Wldmer, F.D. Finkelman, H. Madana, C.A. Jacobs, K.H. Grabstein, and C.R. Maliszewski. 1993. Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses in vivo. *J. Immunol.* 150:2717-2723.

40. Maliszewski, C.R., T.A. Sato, B. Davison, C.A. Jacobs, F.D. Finkelman, and W.C. Fanslow. 1994. In vivo biological effects of recombinant soluble interleukin-4 receptor. *Proc. Soc. Exp. Biol. Med.* 206:233-237.

41. Walsh, G.M., A. Harbell, A.J. Wardlaw, K. Kuritaka, C.J. Sanderson, and A.B. Kay. 1990. IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11/18)-dependent manner. *Immunology.* 71:258-65.

42. Ebsawa, M., M.C. Liu, T. Yamada, M. Kato, I.M. Lichtenstein, B.S. Bochner, and R.P. Schleimer. 1994. Eosinophil transendothelial migration induced by cytokines. II. Potentiation of eosinophil transendothelial migration by eosinophil-active cytokines. *J. Immunol.* 152:4590-4596.

43. Yamaguchi, Y., Y. Hayashi, Y. Sugama, Y. Murata, T. Kasa- hara, S. Kitamura, M. Torsu, S. Mitu, A. Tomingaka, K. Takatsu, and T. Suda. 1988. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. *J. Exp. Med.* 167:1737-1742.

44. Collins, P.D., S. Marleau, D.A. Griffiths-Johnson, P.J. Jose, and T.J. Williams. 1995. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J. Exp. Med.* 182:1169-1174.

45. Jose, P.J., D.A. Griffiths-Johnson, P.D. Collins, D.T. Walsh, R. Moqbel, N.F. Totty, O. Traning, J.J. Huan, and T.J. Williams. 1994. Eotaxin: a potent eosinophil chemotactic cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.* 179:881-887.

46. Gonzalo, J.A., G.Q. Jia, V. Aguiet, D. Friend, A.J. Coyle, N.A. Jenkins, G.S. Lin, H. Katz, A. Lichtman, N. Copeland, M. Kopf, and J.C. Gutierrez-Ramos. 1996. Mouse eotaxin expression parallels eosinophil accumulation during lung allergic inflammation, but it is not restricted to a Th2-type response. *Immunity.* 4:1-14.

47. Gerard, N.P., M.K. Hodges, J.M. Drazen, P.F. Weller, and C. Gerard. 1989. Characterization of a receptor for CS5a anaphylatoxin on human eosinophils. *J. Biol. Chem.* 1989:1760-1766.

48. Brusco, D.M., R.S. Cotran, and J.S. Pober. 1992. Effects of tumor necrosis factor, lipopolysaccharide, and IL-4 on the expression of vascular cell adhesion molecule-1 in vivo. *J. Immunol.* 149:2954-2960.

49. Schleimer, R.P., S.A. Sterbinsky, J. Kaiser, C.A. Buckel, D.A. Klunk, K. Tomioka, W. Newman, F.W. Luscinskas, M.A. Gimbrone, Jr., B.W. McIntyre, and B.S. Bochner. 1992. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. *J. Immunol.* 148:1086-1092.

50. Coffman, R.L., K. Varkila, P. Scott, and R. Chatelain. 1991. Role of cytokines in the differentiation of CD4+ T-cell subsets in vivo. *Immunol. Rev.* 123:189-207.