Cutting Edge: IPSE/alpha-1, a Glycoprotein from *Schistosoma mansoni* Eggs, Induces IgE-Dependent, Antigen-Independent IL-4 Production by Murine Basophils In Vivo

Gabriele Schramm, Katja Mohrs, Maren Wodrich, Michael J. Doenhoff, Edward J. Pearce, Helmut Haas and Markus Mohrs

*J Immunol* 2007; 178:6023-6027; doi: 10.4049/jimmunol.178.10.6023

http://www.jimmunol.org/content/178/10/6023

References

This article cites 24 articles, 9 of which you can access for free at: http://www.jimmunol.org/content/178/10/6023.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Cutting Edge: IPSE/alpha-1, a Glycoprotein from Schistosoma mansoni Eggs, Induces IgE-Dependent, Antigen-Independent IL-4 Production by Murine Basophils In Vivo

Gabriele Schramm, Katja Mohrs, Maren Wodrich, Michael J. Doenhoff, Edward J. Pearce, Helmut Haas, and Markus Mohrs

During infection with the helminth parasite Schistosoma mansoni, the deposition of eggs coincides with the onset of IL-4 production and Th2 development. Although IL-4 is known as a potent inducer of Th2 differentiation, the mechanism by which schistosome eggs induce IL-4 production is not clear. In this study, we demonstrate that the Schistosoma mansoni egg Ag (SmEA) induces IgE-dependent IL-4 production by basophils derived from Heligmosomoides polygyrus-infected or OVA/alum-immunized mice in the absence of pathogen-specific IgE. The effect is mediated by the secretory glycoprotein IPSE/alpha-1, because IPSE/alpha-1-depleted SmEA no longer induces cytokine production. Conversely, recombinant IPSE/alpha-1 is sufficient to induce IL-4 production. Importantly, the injection of SmEA or recombinant IPSE/alpha-1 into H. polygyrus-infected 4get/KN2 IL-4 reporter mice rapidly induces the dose-dependent IL-4 production by basophils in the liver, a major site of egg deposition. Thus, IPSE/alpha-1 induces basophils to produce IL-4 even in the absence of Ag-specific IgE. The Journal of Immunology, 2007, 178: 6023–6027.

Schistosoma mansoni is a helminth parasite that chronically infects men and mice. The infection is acquired during exposure to fresh water cercariae, which burrow through the skin (1). The course of infection in both host species is characterized by an early, moderate Th1 phase followed by a vigorous Th2 response during the acute stage (1). Failure to initiate a Th2 response in both species associated with high-pathology schistosomiasis (2). In fact, IL-4–/– mice succumb to infection with S. mansoni and even the selective deletion of the IL-4Rα in macrophages is detrimental (3, 4). These findings underscore the importance of IL-4 and Th2 immunity for the host and consequently for the survival and propagation of the parasite. The onset of IL-4 production weeks after infection coincides with the deposition of S. mansoni eggs in the liver, and schistosome eggs alone are sufficient to induce Th2 responses in naive mice (1). Although IL-4 is known as a potent inducer of Th2 differentiation, the mechanisms by which S. mansoni eggs and their soluble Ags (SmEA) induce IL-4 production and Th2 development are still elusive. Recently we identified a glycoprotein secreted by S. mansoni eggs, IPSE/alpha-1, as a factor triggering basophils from nonsensitized humans to release IL-4 in vitro when costimulated with IL-3 (5, 6). However, it is not known whether this activity of IPSE/alpha-1 is limited to human basophils and whether basophils can be activated in vivo. In this study we use 4get/KN2 IL-4 dual-reporter mice (7) in which IL-4-competent cells are GFP and IL-4-producing cells additionally express huCD2 to obtain highly purified basophils and visualize IL-4 production directly in vivo. We show that IPSE/alpha-1 is necessary and sufficient to induce IL-4 production by murine basophils in an IgE-dependent, Ag-independent manner. Importantly, we demonstrate that SmEA and recombinant IPSE/alpha-1 induce hepatic basophils to produce IL-4 within hours of i.v. injection. Collectively, our data demonstrate that IPSE/alpha-1 induces IgE-dependent, Ag-independent IL-4 production by murine basophils in vivo, revealing an innate pathway that shows how basophils might direct Th2 development before the generation of Ag-specific IgE.

Materials and Methods
Mice, pathogens, and pathogen derivates
4get (C.129-I[4get2(Ly5.1)]J) (8) and 4get/KN2 (7) IL-4 reporter BALB/c mice were kept under specific pathogen-free conditions at the animal facility of the

1 This work was supported by funds from the Trudeau Institute, National Institutes of Health Grants AI46566 (to M.M.), AI072296 (to M.M.), and AI32573 (to E.J.F.), and the Deutsche Forschungsgemeinschaft Grants SFB/TRR22 (A12) and GRK 288 (to G.S. and H.H.). Schistosome life stages for these experiments were in part provided by the National Institutes of Allergy and Infectious Diseases Contract NO155270.

2 G.S. and K.M. contributed equally to this work.

3 Address correspondence and reprint requests to Dr. Markus Mohrs, Trudeau Institute, 154 Algonquin Avenue, Saranac Lake, NY 12983. E-mail address: mmohrs@trudeauinstitute.org

4 Abbreviations used in this paper: SmEA, Schistosoma mansoni egg Ag; alum, aluminium hydroxide; HEK, human embryonic kidney; Hg, Heligmosomoides polygyrus; WT, wild type.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00
next produce IL-4 whereas anti-IgE or the addition of Hp Ag induced robust IL-4 production. SmEA and both the glycosylated and nonglycosylated forms of recombinant IPSE/alpha-1 also induced robust IL-4 production. In striking contrast, no IL-4 was detected when SmEA was selectively depleted of IPSE/alpha-1 (Fig. 1B). Thus, the IPSE/alpha-1 present in SmEA is a potent inducer of IL-4 production by murine basophils.

Although IPSE/alpha-1 clearly induces basophils to produce IL-4, we cannot exclude the possibility that other IL-4 competent cells, such as Th2 cells or eosinophils, also produce IL-4 in response to this stimulus. This issue was addressed by culturing whole blood cells from Hp-infected 4get/KN2 IL-4 dual-reporter mice in which IL-4-competent cells are GFP+ and IL-4-producing cells additionally express huCD2 (7). Whole blood containing Th2 cells and eosinophils in addition to basophils (7, 15) was taken 2 wk after Hp infection and cultured under the indicated conditions (Fig. 2). As expected (Fig. 1) (7), blood-borne cells from Hp-infected mice did not express huCD2 despite a high frequency of GFP+ cells (Fig. 2B). SmEA induced huCD2 expression expectedly only in GFP+, IL-4-competent cells (Fig. 2A), and only FceRI+ cells (i.e., basophils) expressed huCD2 within the GFP+ population. Thus, the stimulation of murine blood selectively triggered basophils to produce IL-4.

Next, we tested the effects of the same stimuli as used in Fig. 1B on huCD2 expression by basophils ex vivo (Fig. 2B). As expected (Figs. 1B and 2B) (7), unstimulated blood-borne basophils isolated from Hp-infected mice did not express huCD2. Stimulation with SmEA or recombinant IPSE/alpha-1, but not with IPSE/alpha-1-depleted SmEA, induced huCD2 expression. The expression of huCD2 qualitatively and quantitatively reflected IL-4 protein production by purified basophils (Fig. 1B) and extended these observations to the single cell level. Soluble human IgE and IgG have been shown to bind IPSE/alpha-1 Ag nonspecifically, thereby competing out Fc receptor-bound IgGs and suppressing the IL-4-inducing effect of IPSE/alpha-1 (6). To test whether polyclonal murine Igs bind IPSE/alpha-1, we preincubated SmEA, E. coli-IPSE, and Hp Ag with PBS or serum from Hp-infected mice before the addition of whole blood cells from Hp-infected 4get/KN2 mice (Fig. 2C). Sera from mice infected with parasitic nematodes such as Hp contain high IgE and IgG1 concentrations (data not shown) (8, 12). Indeed, the preincubation with Hp immune serum, which
is not known to recognize SmEA, reduced not only the IL-4-
inducing capacity of Hp Ag but also that of SmEA and E.
coli/IPSE, suggesting that IPSE/alpha-1 binds Ag nonspecifi-
cally to murine Igs.

Because infection with Hp might condition basophils to re-
spond to SmEA, we next analyzed basophils isolated from
OVA/alum-immunized mice. Although we have previously
shown that Th2 cells from Hp-infected mice do not produce
IL-4 in response to SmEA and vice versa (7), immunization
with OVA/alum excludes the possibility of IgE Abs directed
against common or cross-reactive Ags shared between
Hp and OVA-specific IgE response was induced by im-
uminating 4get/KN2 mice with OVA/alum (Fig. 3). Compared
with Hp-infected mice, very few basophils were detected in the
blood of OVA/alum-immunized animals (>80% fewer ba-
sophils) and lower FceRI expression was apparent (Fig. 3A). In
contrast to Hp infection (Fig. 2B), some huCD2 expression
was detected on blood-borne basophils of OVA/alum-immun-
ized animals, presumably due to the persistence of OVA Ag (Fig.
3B). However, the addition of SmEA, anti-IgE, or OVA in-
creased huCD2 expression, whereas Hp Ag had no effect. These
data further demonstrate that SmEA-induced IL-4 production
by basophils is not due to Ag-specific IgE and does not require
any preexisting infection, extensive basophilia, or FceRI
up-regulation.

Signaling through FceRI triggers the effector functions of ba-
sophils in Th2 immunity and IgE-mediated responses (11, 13).
Because IPSE/alpha-1 is an IgE-binding factor (6) we tested
whether SmEA-induced basophil IL-4 production is IgE de-
pendent. IgE−/− and FVB WT controls were infected with
Hp and basophils were enriched from the blood. Of note, basophils
cannot be sorted by anti-IgE staining because this results in ac-
tion and IL-4 production (7, 11). Thus, basophils were en-
riched either as Thy1.1dullTCR− cells (14) or by sorting for
CD4+ CD8− CD19− CCC3− NK1.1− TCRγδ− cells (Fig. 4A).
Importantly, both strategies eliminate all blood-borne potential
IL-4 producers (i.e., CD4+ T cells, NK T cells, and eosino-
phil) except basophils (11), and basophils are the only popu-
lation in the blood that produces IL-4 in response to SmEA

![FIGURE 2.](http://www.jimmunol.org/)

**FIGURE 2.** IPSE/alpha-1 selectively induces basophils to produce IL-4. *A*, 4get/KN2 mice were infected with Hp and 2 wk later unseparated blood cells were cultured in the presence of SmEA. Four to six hours later the cells were transferred onto ice, stained with the indicated mAb, and analyzed by FACS. *B*, As in *A* but the cells were cultured in the presence of the indicated reagents. FACS analyses were gated on FceRI+ cells. *C*, As in *A* and *B*, but the indicated stimulants were preincubated for 30 min in the absence (−) or presence (+) of serum from Hp-infected mice before the addition of unseparated blood cells. Numbers in *A* indicate the frequency of cells within each quadrant, and in *B* and *C* they indicate the frequency of huCD2+ cells within the GFP+ population. PI− indicates propidium iodide negative (i.e., live) cells. Data are representative of at least two independent experiments.

![FIGURE 3.](http://www.jimmunol.org/)

**FIGURE 3.** SmEA-induced IL-4 production by basophils is independent of infection, pronounced basophilia, or high FceRI expression. 4get/KN2 mice were infected with Hp or immunized and boosted i.p. with OVA/alum. *A*, After 2 wk (Hp infection) or 4 wk (OVA/alum immunization), respectively, peripheral blood was analyzed by FACS. *B*, Unseparated cells from OVA/alum-im-
munized mice were cultured for 4 h in the presence of the indicated reagents. The cells were then transferred onto ice for subsequent Ab staining. FACS analyses in *B* were gated on FceRI+ cells. Numbers indicate the frequency of huCD2+ cells within the GFP+ population. Data are representative of at least two independent experiments.

![FIGURE 4.](http://www.jimmunol.org/)

**FIGURE 4.** SmEA-induced IL-4 production by basophils is IgE dependent. *A*, FVB WT controls and IgE-deficient mice were infected with Hp. Blood was harvested 2 wk later and stained for CD19-FITC and CD4/CD8α/CCR3/NK1.1/TCRγδ-PE cells (top panels). Small aliquots were subsequently sensi-
tized with IgE, stained with anti-IgE, and analyzed for FceRI expression within the PE+/FITC− population (see gate) to assess the frequency of basophils (lower panel). *B*, FITC+/PE- cells (see gate in top panel of *A*) were sorted by FACS and cultured in the presence of the indicated reagents. The supernatants were harvested 18 h later and analyzed for IL-4 by ELISA. The dotted line indicates the limit of detection. Data are representative of two independent experiments. Bars depict the mean ± SD from triplicate cultures.
Basophils from WT mice produced IL-4 upon stimulation with SmEA or anti-IgE, whereas the same reagents induced no response in the corresponding IgE−/− cultures (Fig. 4B). Importantly, upon basophil-specific stimulation with IL-3 plus IL-18 (7), IL-4 was detected in both cultures, demonstrating that IgE−/− basophils are capable of unimpaired IL-4 production. Thus, IPSE/alpha-1 induces basophils to produce IL-4 via a direct or indirect IgE-dependent mechanism.

Last, we wanted to test the ability of IPSE/alpha-1 in SmEA to induce basophil IL-4 production in vivo. Hp-infected 4get/KN2 mice (7) were injected i.v. with PBS, graded doses of SmEA, or Hp Ag and the liver was analyzed 4 h later (Fig. 5A). As published (7), hepatic basophils were huCD2− in PBS-injected, Hp-infected mice. In contrast, the injection of SmEA induced the dose-dependent expression of huCD2 on hepatic basophils. Because IPSE/alpha-1 is the only component in SmEA activating murine basophils in vitro (Figs. 1 and 2), we set out to test whether IPSE/alpha-1 is also responsible for SmEA-induced basophil IL-4 production in vivo. Hp-infected 4get/KN2 mice were injected i.v. with PBS, SmEA, or IPSE/alpha-1-depleted SmEA from the same batch, and the liver (Fig. 5B) was analyzed 4 h later. Whereas native SmEA induced the expression of huCD2 on basophils, the same amount of IPSE/alpha-1-depleted SmEA induced consistently fewer basophils (<50%) to express huCD2 and the expression levels were consistently lower. Conversely, recombinant HEK-IPSE (Fig. 5C) and E. coli-IPSE (data not shown) were sufficient to induce dose-dependent huCD2 expression.

Although many studies have shown that basophils can be a source of IL-4 during acute infection or T cell-dependent memory responses, little is known about Ag-nonspecific innate responses (6, 14, 16–18). Collectively we demonstrate that S. mansoni IPSE/alpha-1 induces murine basophils in an IgE-independent, Ag-independent manner to rapidly produce IL-4. The activity of IPSE/alpha-1 is not contingent on the preconditioning of basophils by preceding infections (Fig. 3). Importantly, IL-4 production can be induced in basophils in the liver, a major site of S. mansoni egg deposition. Although the present study does not assess the effect of IPSE/alpha-1 and SmEA on Th2 differentiation, IL-4 is known as a key factor for Th2 development (8, 19). Of note, it has previously been demonstrated that T cell responses can be primed locally in the liver (20, 21). As we demonstrate in the present study, the IgE-dependent, Ag-nonspecific activation of basophils constitutes an innate mechanism for S. mansoni eggs to induce IL-4 production even before the generation of S. mansoni-specific IgE or any preexisting infection. Although basophils produce less IL-4 in response to Ag-nonspecific IPSE/alpha-1 stimulation as compared with Ag-specific stimulation, the innate release of IL-4 is likely to precede Th2 priming mediated by dendritic cells. Moreover, IL-4 increases the Ag-presenting and costimulatory potential of dendritic cells and is commonly used to mature bone marrow-derived dendritic cells (22). Therefore, early basophil-derived IL-4 can potentially foster Th2 development in two ways: directly as a factor inducing Th2 development, and indirectly by maturing dendritic cells for optimal Ag presentation and Th2 priming. These observations are consistent with a model whereby basophil-derived IL-4 might link innate responses to subsequent Th2 development (23, 24). It is tempting to speculate that other type 2-inducing pathogens have evolved to produce similar substances that innately activate basophils, thereby jump-starting IL-4 production and Th2 immunity.

Acknowledgments

We thank Dr. F. Finkelman for providing IgE−/− mice and FVB WT controls, S. Monard and B. Sells for cell sorting, D. Duuo for technical assistance, and Dr. F. Lund for critical review of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References


