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Cutting Edge: Helminth Infection Induces IgE in the Absence of μ- or δ-Chain Expression[1]

Georgia Perona-Wright,* Katja Mohrs,* Justin Taylor,† Colby Zaph,† David Artis,† Edward J. Pearce,† and Markus Mohrs[2]*

Infections with helminth parasites are associated with an IgE isotype switch and high serum IgE concentrations. IgE is rapidly bound by the high affinity IgE receptor (FcεRI), thereby sensitizing FcεRI-bearing basophils and mast cells for IgE-inducible effector functions such as IL-4 production. The development of Ab-secreting B cells is dependent on IgM and consequently, μMT mice, which lack surface IgM, are considered devoid of Abs. In this study we report the unexpected finding that C57BL/6 μMT mice generate robust IgE responses upon infection with three distinct helminth parasites, *Heligmosomoides polygyrus*, *Trichuris muris*, and *Schistosoma mansoni*. IgE is produced despite an apparent block in B cell development and licenses basophils for IgE-induced IL-4 production. Our findings reveal the existence of an evolutionarily conserved, IgM-independent pathway for the production of IgE upon infection with helminth parasites. ***The Journal of Immunology,*** **2008, 181: 6697–6701.**

Distant host species ranging from mouse to man mount robust IgE responses to diverse helminth parasites, indicating that the induction of IgE is an evolutionarily conserved immune mechanism. A key function of IgE is the sensitization of cells that express the high affinity IgE receptor FcεRI. This licenses FcεRI-bearing cells, such as basophils, to respond to IgE-mediated stimuli (1), enabling various effector functions critical to host defense including the production of IL-4 (1–5). Interestingly, even nonspecific IgE can elicit basophil IL-4 when engaged by soluble egg Ag of the helminth parasite *Schistosoma mansoni* (3). Moreover, even monomeric IgE enhances the survival and expansion of FcεRI-bearing cells and increases their surface FcεRI expression, creating a positive feedback loop that amplifies their sensitivity to IgE-mediated stimulation (1, 6–9).

The development of B cells capable of secreting Ab is dependent on surface expression of IgM, and consequently μMT mice, which lack surface IgM and IgD, are considered devoid of Abs (10). However, μMT mice on a BALB/c background show an incomplete block in B cell development, and the presence of both serum Ig and mature B cells has been reported (11, 12). In contrast, it is generally accepted that normal B cell maturation is fully inhibited in C57BL/6 μMT animals. Nonetheless, IgA has been reported in these mice, leading to the suggestion of an evolutionary primitive system in which immature B cells switch directly to IgA production. Of note, no other isotypes, including IgE, were detected (13).

In this study we report that μMT mice on a C57BL/6 background generate robust and immunologically functional IgE responses upon infection with three distinct helminth parasites. Our data reveal an unsuspected IgM-independent pathway for the production of IgE.

Materials and Methods

Mice, infections, and immunization

4get (C.129-*Il4*^{tm1Lky}/J) (14) mice were backcrossed to the C57BL/6 genetic background for 10 generations. All mice, including μMT (10) and $J_H^{-/-}$ (15), were bred and housed at Trudeau Institute (Saranac Lake, NY). All animals were kept under specific pathogen-free conditions and were used at 8–12 wk of age. Mice were inoculated by gavage with 200 *Heligmosomoides polygyrus* (*Hp*)[3] larvae, 200–400 embryonated *Trichuris muris* (*Tm*) eggs, or exposed percutaneously to 50 *S. mansoni* (*Sm*) cercariae as described (3, 16, 17). Two hundred *Hp* larvae were used for s.c. immunization. OVA/alum immunization was performed as described (3). All experimental procedures with mice were approved by the Institutional Animal Care and Use Committee of the Trudeau Institute and the University of Pennsylvania.

ELISA and ELISPOT

Serum IgE concentrations were quantified by ELISA with the paired mAbs R35-72 and R35-118 using murine IgE as a standard. The same Abs were used for ELISPOT assays; MultiScreen hemagglutinin filter plates (Millipore) were coated overnight at 4°C, blocked with FBS, and incubated with cells overnight at 37°C. IgE secretion was detected with a streptavidin-alkaline phosphatase conjugate and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/NBT substrate (Sigma-Aldrich). IL-4 in culture supernatant was quantified as described (2).

Flow cytometry and cell sorting

The following mAbs against mouse Ags were used as PE, allophycocyanin, or biotin conjugates: CCR3 (83101), CD4 (RM4-5), CD19 (6D5), CD25 (PC61), CD43 (S7), CD45R (B220; RA3-6B2), CD90.2 (Thy1.2; 53-2.1),

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[3] Abbreviations used in this paper: *Hp*, *Heligmosomoides polygyrus*; mesLN, mesenteric lymph node; *Sm*, *Schistosoma mansoni*; *Tm*, *Trichuris muris*; WT, wild type.

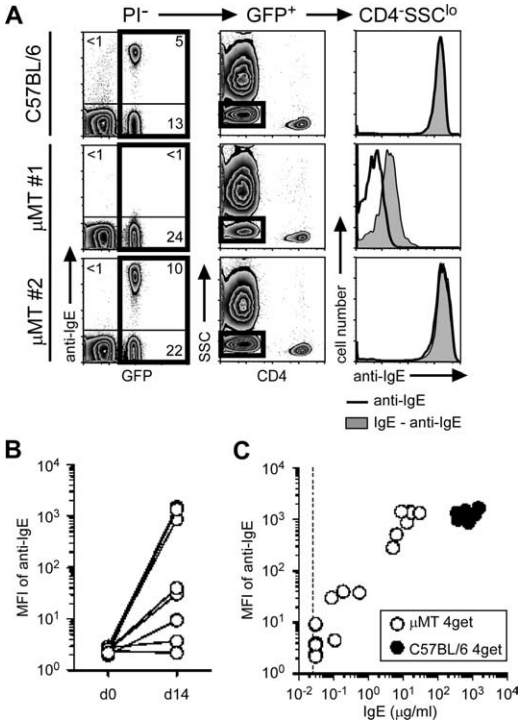

FIGURE 1. IgE production in the absence of IgM or IgD. *A*, μMT 4get (two representative mice) and C57BL/6 WT 4get mice were infected with *Hp* and 2 wk later PBL were analyzed by FACS for surface IgE. The presence of FcεRI on basophils was demonstrated by in vitro IgE sensitization (histogram overlays). *B*, Individual μMT 4get mice shown before and 2 wk after infection. *C*, IgE staining in correlation to serum IgE concentrations, as determined by ELISA, 2 wk after infection. Dashed line indicates the limit of detection.

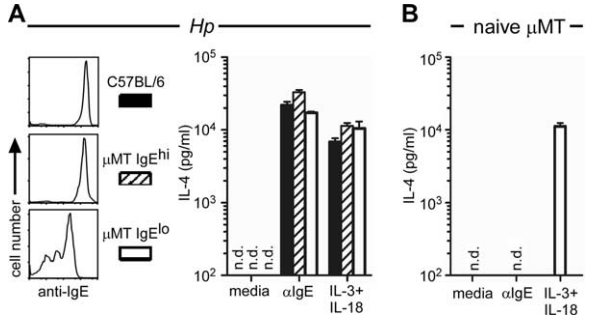

FIGURE 2. IgE in μMT mice is immunologically active. *A*, μMT 4get and C57BL/6 WT 4get mice were infected with *Hp* and basophils were sorted from the indicated groups. The purified cells were stimulated in triplicates for 24 h and the production of IL-4 was determined by ELISA. *B*, Basophils from naive μMT 4get mice were sorted and stimulated as in *A*. n.d. Not detectable; αIgE, anti-IgE.

and IgE (R35-72 and R35-118). Additional reagents included streptavidin-PE, streptavidin-allophycocyanin, and mouse anti-DNP IgE (SPE-7). Surface staining with mAb, acquisition, and analyses were performed as described (3, 14). To sort basophils, *Hp*-infected mice were bled on day 12 and grouped by above or below average surface IgE staining on basophils into IgE^{high} and IgE^{low}. On day 14 PBL were pooled within groups, an aliquot was stained for IgE, and $GFP^+CCR3^-CD4^-$ cells were sorted and cultured as described (3). $B220^+$ and $B220^-$ cells were sorted from mesenteric LN cells from day-14 *Hp*-infected mice.

Results and Discussion

Infection-induced IgE production in the absence of IgM and IgD

Staining for FcεRI-bound IgE on the surface of basophils is an immunologically relevant method for the measurement of IgE in vivo (2, 3, 8) that is more sensitive than standard serum ELISA techniques. Basophils can be identified in the blood of 4get IL-4 reporter mice as GFP^+ and either $CD4^-SSC^{low}$ or $CD4^-CCR3^-$ (2, 3, 14). 4get and 4get.μMT mice, both on a C57BL/6 background, were infected with the helminth parasite *Hp* and 2 wk later PBL were stained with anti-IgE to detect surface-bound IgE. As expected (2, 3), strong IgE staining was observed on GFP^+ cells in *Hp*-infected wild-type (WT) mice (Fig. 1*A*, *left column*). Surprisingly, however, IgE staining was also apparent in the vast majority of *Hp*-infected μMT mice (126 IgE^+ of 154 *Hp*-infected μMT mice in 14 independent experiments). Identical results were obtained in C57BL/6 μMT mice that were not on the 4get background (see Fig. 3*B*). Importantly, the production of IgE in μMT mice was only observed in infected but not in naive animals (Figs. 1*B* and 2*B*).

The minority of *Hp*-infected μMT mice without detectable IgE retained both basophils, identified as $GFP^+CD4^-SSC^{low}$ cells, and the ability to bind IgE, as demonstrated by in vitro sensitization with IgE (Fig. 1*A*, *middle* and *right columns*, respectively) (2, 3). The intensity of IgE staining after in vitro sensitization was low in these animals, consistent with basal FcεRI expression in the absence of IgE-induced up-regulation (8). The heterogeneity of IgE staining between individual mice correlated with serum IgE concentration (Fig. 1*C*) (8).

IgE in μMT mice confers immunological function

The production of IL-4 upon engagement of FcεRI-bound IgE is a hallmark of basophil function (1–3, 18). To test whether the IgE in *Hp*-infected μMT mice confers immunological function, we isolated basophils ($GFP^+CD4^-CCR3^-$) from these animals and measured the production of IL-4 in response to anti-IgE stimulation. *Hp*-infected μMT mice were bled before terminal PBL collection and categorized based on basophil IgE staining into an IgE^{high} and an IgE^{low} pool. Although IgE staining on the IgE^{high} pool was homogeneous and comparable to that of the WT control group, the IgE^{low} pool displayed substantial heterogeneity and a markedly reduced mean fluorescence intensity (Fig. 2, *left panels*). Upon stimulation with anti-IgE, all three cultures released similar amounts of IL-4 (Fig. 2*A*). As expected, IL-4 was undetectable in unstimulated cultures whereas the IgE-independent yet basophil-specific stimulation with IL-3 plus IL-18 also elicited IL-4 from all groups (3, 19). Consistent with the absence of IgE in naive μMT mice (Fig. 1*B*), basophils sorted from these animals did not produce IL-4 upon stimulation with anti-IgE but did respond to IL-3 plus IL-18 (Fig. 2*B*). These data demonstrate that IgE is not present in naive μMT mice, whereas even small amounts of IgE in *Hp*-infected μMT confer immunological function.

IgE production occurs despite a sustained block in B cell development

It has previously been shown that μMT mice on the BALB/c, but not C57BL/6, background display an incomplete block in B cell development and harbor mature B cells in secondary lymphoid organs (11, 12). Although all μMT animals used here were on a C57BL/6 background, we considered the possibility that *Hp* infection might overcome the B cell developmental block (10–12). $CD19^+B220^+$ B cells were not detected in the mesenteric lymph nodes (mesLN), PBL, spleen, or peritoneal cavity of *Hp*-infected μMT mice, whereas they were abundant in C57BL/6 WT controls (Fig. 3*A* and data not shown). The

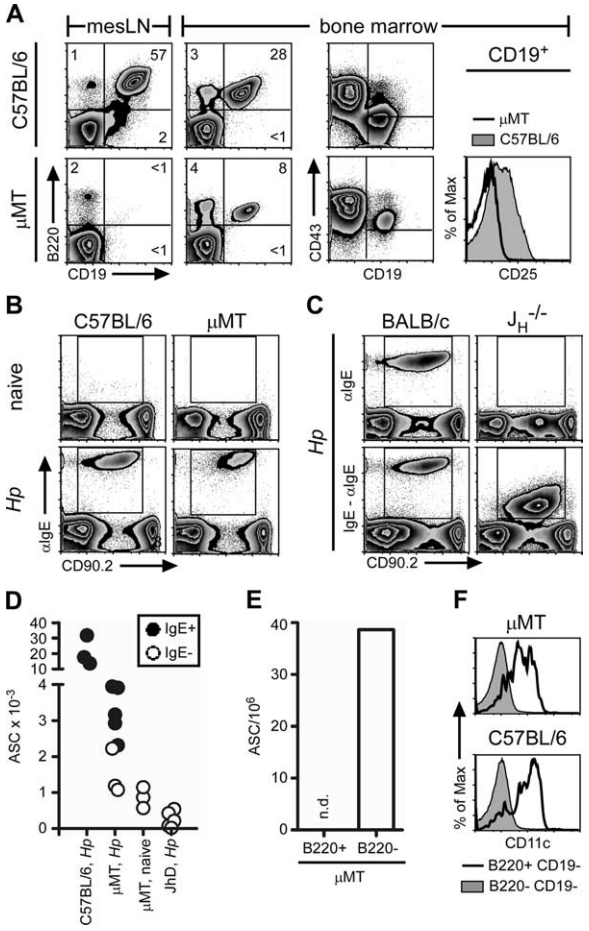

FIGURE 3. IgE-secreting cells are present despite a sustained block in B cell development. *A*, μMT mice and C57BL/6 WT controls were infected with *Hp* and analyzed 2 wk later by FACS. *B*, PBL of naive or 2-wk *Hp*-infected non-4get μMT mice and C57BL/6 WT controls as analyzed by FACS. *C*, PBL of B cell-deficient $J_H^{-/-}$ mice and BALB/c WT controls analyzed by FACS 2 wk postinfection, with or without prior IgE sensitization. *D*, Naive or 2 wk *Hp*-infected mice were analyzed for basophil-bound IgE by FACS and by ELISPOT for IgE-secreting cells (ASC) in the mesLN. *E*, $B220^+$ and $B220^-$ cells (see *A*) were sorted from the mesLN of *Hp*-infected μMT mice and analyzed as in *D*. n.d. Not detectable. *F*, μMT and WT mice were infected as in *A* and the specified populations in the mesLN were analyzed for CD11c expression.

absence of B cells in the peritoneal cavity suggests that B-1 cells are not the source of IgE in infected μMT mice (10, 20). Consistent with a developmental arrest at the stage of pre-B cell maturation, $CD19^+$ cells in the bone marrow of μMT mice failed to down-regulate CD43, did not induce CD25, and were present at low frequency (Fig. 3*A*, *right panels*) (10, 11, 13). As expected neither surface IgM nor IgD was detected in μMT mice even after *Hp* infection (data not shown) (10, 13).

Given the ready detection of IgE in infected C57BL/6 μMT mice, we examined another B cell-deficient strain, $J_H^{-/-}$ mice (15). While μMT mice lack surface expression of the μ- and δ-chains, $J_H^{-/-}$ mice carry a deletion of the J_H gene segment (10, 15). In non-4get mice, basophils can be identified by their $Thy1^{dull}$ phenotype in combination with IgE staining (Fig. 3*B*) (3, 21). Consistent with 4get μMT mice (Fig. 1), we observed robust IgE staining of $Thy1^{dull}$ cells in *Hp*-infected, but not naive, C57BL/6 μMT mice that were not on the 4get background (Fig. 3*B*). In contrast, no IgE was detected in *Hp*-infected

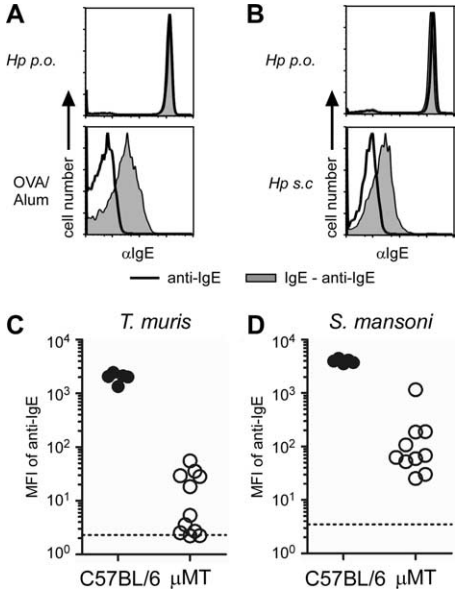

FIGURE 4. Diverse helminth parasites induce IgE in μMT mice. *A*, μMT 4get mice were infected per os (p.o.) with *Hp* or immunized i.p. with OVA/alum and basophils in the PBL were analyzed for IgE. FcεRI expression on basophils was demonstrated by in vitro IgE sensitization. *B*, μMT 4get mice either infected p.o. or immunized s.c. with *Hp* larvae and analyzed as in *A*. αIgE, anti-IgE. *C* and *D*, μMT 4get and C57BL/6 WT 4get mice were infected with *Tm* (*C*) or *Sm* (*D*) and PBL basophils were analyzed for IgE 3 (*C*) or 8 wk (*D*) later. Dashed lines represent anti-IgE staining on CD4-gated cells, which are devoid of FcεRI.

$J_H^{-/-}$ animals despite a clear $Thy1^{dull}$ basophil population able to bind IgE upon in vitro sensitization (Fig. 3*C*). These data demonstrate that the J_H gene segment is required for infection-induced IgE production whereas the μ- and δ-chains are dispensable.

Because *Hp*-infected μMT mice produce immunologically functional IgE but no mature B cells could be identified by FACS (Fig. 3*A*), we used an ELISPOT assay as a functional readout to detect IgE-producing cells from the mesLN, spleen, and bone marrow of *Hp*-infected C57BL/6 WT, μMT, and $J_H^{-/-}$ mice. The number of IgE-secreting cells in the mesLN of *Hp*-infected μMT mice was markedly increased above the background of naive controls (Fig. 3*D*). Substantially fewer or no IgE-secreting cells were found in μMT mice that were negative for IgE on basophils by FACS analysis. No IgE-secreting cells were detected in the spleen or bone marrow (data not shown) or in the mesLN of *Hp*-infected $J_H^{-/-}$ animals (Fig. 3*D*). Separating mesLN cells from *Hp*-infected μMT mice based on the expression of B220 (Fig. 3*A*) identified $B220^-$ cells as the source of IgE (Fig. 3*E*). The $B220^+CD19^-$ population in both μMT and C57BL/6 mice expressed CD11c, consistent with a plasmacytoid dendritic cell phenotype (Fig. 3*F*).

IgE production in μMT mice requires productive infection with diverse helminth parasites

Aluminum hydroxide (alum) is widely used as an adjuvant to elicit Th2 responses and the production of IgE (3). To test whether alum is sufficient to elicit IgE in μMT mice, we primed and boosted with OVA/alum and compared the IgE response to that induced by *Hp* infection. No IgE was detected on basophils isolated from OVA/alum-immunized C57BL/6 μMT mice, whereas the majority of *Hp*-infected μMT mice displayed high

levels of IgE staining (Fig. 4*A*). Furthermore, IgE staining on basophils from OVA/alum-immunized mice remained low upon in vitro IgE sensitization, confirming the in vivo absence of IgE (8). These data suggest that infection with *Hp* larvae, and not any Th2 response, has the distinctive ability to induce IgE production in μMT mice. To determine whether the induction of IgE in μMT mice requires *Hp* larvae to establish productive infection in the gastrointestinal tract or whether the Th2-inducing capacity of *Hp* larvae alone is sufficient (22), we immunized μMT and C57BL/6 WT mice s.c. with *Hp* larvae. This regimen results in a potent Th2 response in the draining lymph node of both WT and μMT mice (data not shown) (22), but larval development is aborted and no intestinal infection occurs. No IgE was detected in μMT mice immunized s.c. with *Hp*, whereas high IgE staining was observed in WT controls (Fig. 4*B*). As in μMT mice challenged with OVA/alum, those immunized with *Hp* s.c. revealed only low levels of IgE staining upon in vitro sensitization, confirming the in vivo absence of IgE (8).

These data suggested that the production of IgE in μMT mice requires productive infection with a helminth pathogen. To explore this further, we infected μMT mice with another murine helminth parasite, *Tm*. In contrast to *Hp*, which establishes persistent infection in the small intestine, *Tm* infects the large intestine and is rapidly expelled (16). As shown in Fig. 4*C*, *Tm* infection also triggered IgE production in μMT mice. However, IgE was not detected in all *Tm*-infected animals, and IgE staining on basophils was lower than that in WT controls. These observations are consistent with the weaker Th2 response elicited by *Tm* than by *Hp* (data not shown) (16, 18). Next we infected C57BL/6 μMT mice and WT controls with *Sm*, a human parasite that nonetheless establishes productive and chronic infection in mice (17, 23). Although *Sm* resides mainly in the hepatic vasculature, eggs are continuously shed through the gastrointestinal wall. All μMT mice produced IgE upon infection with *Sm*, albeit at a lower level than in WT controls (Fig. 4*D*). These experiments demonstrate that IgE is elicited in μMT mice in response to infection with three distinct helminth parasites. Interestingly, all of these helminth parasites affect the gastrointestinal tract, and ectopic immunization of μMT mice with *Hp* did not result in the production of IgE. These observations are consistent with the notion that immature B cells can exit the bone marrow before terminal differentiation and receive switching signals in peripheral sites, a process that may preferentially occur in gut-associated tissue (13).

Collectively, our data demonstrate that surface expression of the Ig μ- and δ-chains is dispensable for the production of IgE upon infection with three distinct helminth parasites. Although the arrest in B cell development can be overcome in μMT mice on the BALB/c background, we have no evidence for a similar event in C57BL/6 μMT mice (11, 12). However, in vitro studies have shown that a direct εH chain isotype switch can occur in precursor B cells in the absence of surface IgM (24). The detection of IgE in helminth-infected μMT mice likely demonstrates the relevance of this pathway in vivo. Indeed, IgE-bearing lymphocytes have been detected in a patient with X-linked agammaglobulinemia (25). Interestingly, B cell development can progress in μMT mice in the absence of the proapoptotic Fas molecule or as a consequence of forced expression of the survival factor Bcl-2 (26, 27). IL-4 was originally identified as a B cell growth and survival factor (4, 28), and thus the production of IL-4 upon helminth infection might link B cell survival with the production of IgE even in the absence of surface IgM (28). Consistent with this, *Hp*-infected IL-4Rα$^{-/-}$ mice produce substantially less IgE than *Hp*-infected μMT mice despite the presence of B cells (data not shown).

IgE enhances the expansion and survival of FcεRI-bearing cells, and sensitization with IgE licenses these cells for IgE-mediated effector functions (1, 6, 7). The ability of diverse helminth parasites to elicit IgE production in the apparent absence of mature B cells suggests the engagement of an ancient, evolutionarily conserved mechanism to ensure the availability of IgE. The recent report of T cell-independent IgE production further supports the existence of such a primitive pathway (29). Importantly, based on our data, helminth-infected μMT mice can no longer be considered devoid of IgE. This has important implications for studies of helminth infections in μMT mice, which are widely used to study the role of IgE in type 2 immune responses.

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Disclosures

The authors have no financial conflict of interest.

References

1. Kawakami, T., and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2: 773–786.
2. Gessner, A., K. Mohrs, and M. Mohrs. 2005. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J. Immunol.* 174: 1063–1072.
3. Schramm, G., K. Mohrs, M. Wodrich, M. J. Doenhoff, E. J. Pearce, H. Haas, and M. Mohrs. 2007. Cutting Edge: IPSE/α-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *J. Immunol.* 178: 6023–6027.
4. Finkelman, F. D., T. Shea-Donohue, J. Goldhill, C. A. Sullivan, S. C. Morris, K. B. Madden, W. C. Gause, and J. F. Urban, Jr. 1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol.* 15: 505–533.
5. Min, B., M. Prout, J. Hu-Li, J. Zhu, D. Jankovic, E. S. Morgan, J. F. Urban, Jr., A. M. Dvorak, F. D. Finkelman, G. LeGros, and W. E. Paul. 2004. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* 200: 507–517.
6. Kalesnikoff, J., M. Huber, V. Lam, J. E. Damen, J. Zhang, R. P. Siraganian, and G. Krystal. 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 14: 801–811.
7. Asai, K., J. Kitaura, Y. Kawakami, N. Yamagata, M. Tsai, D. P. Carbone, F. T. Liu, S. J. Galli, and T. Kawakami. 2001. Regulation of mast cell survival by IgE. *Immunity* 14: 791–800.
8. Lantz, C. S., M. Yamaguchi, H. C. Oettgen, I. M. Katona, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. IgE regulates mouse basophil FcεRI expression in vivo. *J. Immunol.* 158: 2517–2521.
9. Yamaguchi, M., C. S. Lantz, H. C. Oettgen, I. M. Katona, T. Fleming, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med.* 185: 663–672.
10. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* 350: 423–426.
11. Hasan, M., B. Polic, M. Bralic, S. Jonjic, and K. Rajewsky. 2002. Incomplete block of B cell development and immunoglobulin production in mice carrying the μMT mutation on the BALB/c background. *Eur. J. Immunol.* 32: 3463–3471.
12. Orinska, Z., A. Osiak, J. Lohler, E. Bulanova, V. Budagian, I. Horak, and S. Bulfone-Paus. 2002. Novel B cell population producing functional IgG in the absence of membrane IgM expression. *Eur. J. Immunol.* 32: 3472–3480.
13. Macpherson, A. J., A. Lamarre, K. McCoy, G. R. Harriman, B. Odermatt, G. Dougan, H. Hengartner, and R. M. Zinkernagel. 2001. IgA production without μ or δ chain expression in developing B cells. *Nat. Immunol.* 2: 625–631.
14. Mohrs, M., K. Shinkai, K. Mohrs, and R. M. Locksley. 2001. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* 15: 303–311.
15. Chen, J., M. Trounstine, F. W. Alt, F. Young, C. Kurahara, J. F. Loring, and D. Huszar. 1993. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the JH locus. *Int. Immunol.* 5: 647–656.
16. Zaph, C., K. A. Rook, M. Goldschmidt, M. Mohrs, P. Scott, and D. Artis. 2006. Persistence and function of central and effector memory CD4$^+$ T cells following infection with a gastrointestinal helminth. *J. Immunol.* 177: 511–518.

17. Taylor, J. J., M. Mohrs, and E. J. Pearce. 2006. Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population. *J. Immunol.* 176: 5839–5847.
18. Mohrs, K., A. E. Wakil, N. Killeen, R. M. Locksley, and M. Mohrs. 2005. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity* 23: 419–429.
19. Yoshimoto, T., H. Tsutsui, K. Tominaga, K. Hoshino, H. Okamura, S. Akira, W. E. Paul, and K. Nakanishi. 1999. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc. Natl. Acad. Sci. USA* 96: 13962–13966.
20. Hardy, R. R. 2006. B-1 B cell development. *J. Immunol.* 177: 2749–2754.
21. Voehringer, D., K. Shinkai, and R. M. Locksley. 2004. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 20: 267–277.
22. Ekkens, M. J., Z. Liu, Q. Liu, J. Whitmire, S. Xiao, A. Foster, J. Pesce, J. VanNoy, A. H. Sharpe, J. F. Urban, and W. C. Gause. 2003. The role of OX40 ligand interactions in the development of the Th2 response to the gastrointestinal nematode parasite *Heligmosomoides polygyrus*. *J. Immunol.* 170: 384–393.
23. Pearce, E. J., and A. S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* 2: 499–511.
24. Rolink, A., F. Melchers, and J. Andersson. 1996. The SCID but not the RAG-2 gene product is required for Sμ-Sϵ heavy chain class switching. *Immunity* 5: 319–330.
25. Gajl-Peczalska, K. J., M. Ballow, J. A. Hansen, and R. A. Good. 1973. IgE-bearing lymphocytes and atopy in a patient with X-linked infantile agammaglobulinaemia. *Lancet* 1: 1254.
26. Melamed, D., E. Miri, N. Leider, and D. Nemazee. 2000. Unexpected autoantibody production in membrane Ig-μ-deficient/lpr mice. *J. Immunol.* 165: 4353–4358.
27. Tarlinton, D. M., L. M. Corcoran, and A. Strasser. 1997. Continued differentiation during B lymphopoiesis requires signals in addition to cell survival. *Int. Immunol.* 9: 1481–1494.
28. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T cell-derived B cell growth factor distinct from interleukin 2. *J. Exp. Med.* 155: 914–923.
29. McCoy, K. D., N. L. Harris, P. Diener, S. Hatak, B. Odermatt, L. Hangartner, B. M. Senn, B. J. Marsland, M. B. Geuking, H. Hengartner, et al. 2006. Natural IgE production in the absence of MHC Class II cognate help. *Immunity* 24: 329–339.