Interleukin (IL)-4-independent Immunoglobulin Class Switch to Immunoglobulin (Ig)E in the Mouse

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Summary

Immunoglobulin (Ig) class switching in B cells is regulated by stimuli transduced by cytokines and cell-cell contact. Among these stimuli, interleukin (IL)-4 has been considered an absolute prerequisite for class switching to IgE in the mouse. Here we report that IL-4-deficient (IL-4 −/−) and wild-type mice had comparably elevated serum IgE levels during the course of a murine retrovirus-induced immunodeficiency syndrome, MAIDS. IgE switching in IL-4 −/− mice was also induced by injection of anti-IgD antibody. Treatment with anti-IgD induced germline epsilon (ge) transcripts with comparable efficiency in IL-4 −/− mice and controls, but the levels of productive epsilon transcripts (pe) were lower by a factor of 200 and serum IgE levels were lower by a factor of 300 in IL-4 −/− mice as compared with controls. Induction of ge after anti-IgD treatment of IL-4 −/− mice was unaffected by simultaneous treatment with monoclonal antibodies to IL-4 and IL-4 receptor α chain. Infection of IL-4 −/− mice with Nippostrongylus brasiliensis, a potent stimulus for IgE production, resulted in induction of ge transcripts; however, pe transcripts were barely detectable and serum IgE was not detected. These findings establish a novel IL-4-independent pathway for IgE switching in the mouse that is strongly activated in retroviral infection but weakly in nematode infection. This pathway appears to be dependent on distinct factors that separately control induction of ge transcription and switch recombination to pe.

Ig class switching during an immune response is controlled by cytokines and cognate interactions between T and B cells. For example, IL-4 in conjunction with CD40-CD40L interactions directs class switching to both IgG1 and IgE in the mouse (1, 2). In vitro studies have shown that cytokines regulate germline heavy chain constant region (Cμ) transcription, which is believed to target switch recombination to specific heavy chain isotypes (3, 4). In the mouse, IL-4 stimulates induction of gμ and germline epsilon (ge) transcripts. IFN-γ stimulates increased levels of gγ2a and gγ3 transcripts, and TGF-β stimulates increased levels of gγ2b and gα transcripts. The induction of distinct germline transcripts by different cytokines is reflected in the prevailing serum Ig isotypes during immune responses associated with either type 1 or type 2 cytokine production. Infections eliciting a dominant humoral response feature high-level expression of type 2 cytokines and are associated with elevated serum IgE and IgG1. Delayed-type hypersensitivity responses exhibit high IFN-γ and IL-2 expression and are associated with high levels of serum IgG2a (for review, see reference 5).

It has been shown in vitro and in one in vivo experimental system (injection of goat anti-mouse IgD [GaMδ]) that the switch to IgE in mice is strictly dependent on IL-4, with IL-4 being required to induce increased levels of ge transcripts (6–8). Inhibition of increases in ge transcripts by

1 Abbreviations used in this paper: Cμ, heavy chain constant region; DC-PCR, digestion-circularization PCR; GaMδ, goat anti-mouse IgD; ge, germline epsilon; HPRT, hypoxanthine phosphoribosyl transferase; MAIDS, murine acquired immunodeficiency syndrome; MuLV, murine leukemia virus; pe, productive epsilon; RT-PCR, reverse-transcription PCR.
IL-4 antagonists is associated with failure to induce productive epsilon (pe) transcripts and secretion of IgE during a primary immune response (9, 10). Moreover, mice in which the IL-4 gene has been inactivated by targeted gene disruption failed to produce serum IgE after infection with the nematode *Nippostrongylus brasiliensis* (11, 12).

During our studies of a murine acquired immunodeficiency syndrome (MAIDS), we found that mice deficient for IL-4 and their control littermates produced high levels of serum IgE in response to this retrovirus infection. MAIDS is characterized by increasingly severe immunodeficiency, progressive lymphoproliferation, and hypergammaglobulinemia that develops after infection of susceptible mice with the LP-BM5 mixture of murine leukemia viruses (MuLVs) (13, 14).

To determine whether the phenomenon of IgE switching in the absence of IL-4 was restricted to mice with MAIDS, we took advantage of two well-characterized systems known to induce efficient switching to IgE in vivo: treatment of mice with GaM8 antiserum, and infection with *N. brasiliensis*. In this paper, we report that IL-4 is not required for induction of ge transcripts in either of these systems and that IL-4-deficient mice produced ge transcripts at levels comparable to those of controls after treatment with GaM8; however, germline transcription of e did not necessarily lead to switch recombination at this locus. Thus, even though IL-4−/− mice infected with *N. brasiliensis* produced ge transcripts, they generally operated very low levels of pe transcripts and failed to switch to IgE. IL-4−/− mice treated with anti-IgD, however, did switch to pe and produced serum IgE, but much less than wild-type mice. Only upon retroviral infection were serum IgE levels in the IL-4−/− mice comparable to those found in controls. These findings establish a novel IL-4-independent pathway for IgE switching in the mouse, with possibly distinct factors controlling transcriptional activation, switch recombination, and secretion of IgE.

**Materials and Methods**

**Mice and Viruses.** 129/Ola IL-4−/− (11) were backcrossed to C57BL/6j (B6) for 12 generations (C57BL/6-IL-4−/−Cg129 = B6.IL-4−/−129). Genetically pure B6 IL-4−/− mice (C57BL/6-IL-4−/−Cg129 = B6.IL-4−/−129; Nohin-Trauth, N., manuscript in preparation) were generated from the B6 embryonic stem cell line B6-III (15). B6 and BALB/c female mice were purchased from the Small Animals Division of the National Cancer Institute (Frederick, MD) or from The Jackson Laboratory (Bar Harbor, ME) and were used at 8–12 wk of age.

Mice were inoculated intraperitoneally with 0.1 ml of LP-BM5 MuLV virus pools at 6–8 wk of age. Virus stocks were prepared from the G6 clone of chronically infected SC-1 cells as described previously (16). These stocks contain a mixture of nonpathogenic ecotropic and mink cell focus-inducing MuLV and a disease-causing defective genome. At selected times after infection, mice were killed and bled; serum was stored at −20°C until use. Spleen weight, degree of lymphadenopathy, histopathological evaluations of selected tissues obtained at autopsy, FACS® profiles of splenic cell populations, and in vitro proliferative responses and cytokine production to B and T cell mitogens were used to stage the progression of MAIDS by criteria detailed previously (17–19).

**Antibodies.** Neutralizing mAbs for mouse IL-4 (11B11) (20), GaM8 (21), and rat anti-mouse IL-4R α chain (M1) (22) were as described previously. mAb GL117 (anti-IFN-γ mAb, 2 mg/wk) was used as an isotype control for treatment with M1.

**Stimulation of IgE Responses In Vivo.** Primary IgE responses were induced by injecting mice subcutaneously with a previously determined optimal dose of GaM8 antisera. Antibodies against IL-4 and IL-4R α chain were injected intraperitoneally 24 h before treatment with GaM8 antisera.

**Infection of Mice with N. brasiliensis.** Normal B6, B6.IL-4−/−, and B6.IL-4−/− mice were infected with 500 larvae of *N. brasiliensis* as previously described (5). Some mice were treated with anti-IFN-γ mAb (XGM1.2; 2 mg/wk) or anti-IL-4 mAb (5 mg/wk) at the time of infection. Animals were bled at 14 and 21 d after infection for determinations of serum IgE levels. Secondary challenges with *N. brasiliensis* were given at 3 wk after the primary infection and treatments with anticytokine antibodies were continued. IgE levels were determined in sera obtained at 14 and 21 d after secondary infection.

**Measurement of Polyclonal Serum IgE and IgG1 Levels.** Serum IgE concentrations were measured by ELISA as described previously (23). Briefly, 96-well microtiter plates (Costar Corp., Cambridge, MA) were coated with a mixture (1 μg/ml of each) of the 02131D anti-IgE antibody (PharMingen, La Jolla, CA) and the LO-MA anti-IgE antibody (BioSource, Camarillo, CA). After blocking the plates with BSA and overnight incubation with the samples, the plates were developed using horseradish peroxidase-conjugated goat anti-IgE antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL). Plates were developed with ABTS peroxidase substrate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). IgG1 titers were determined similarly using the appropriate coating and detecting antibodies from Southern Biotechnology Associates, Inc. All ELISA incubations were performed in 10% FCS/PBS.

IgE titers in the sera of LP-BM5 MuLV-infected mice were verified in two other independent ELISA assays for IgE used at DNAAX Research Institute of Molecular and Cellular Biology and in University of Cologne, respectively. To be able to compare IgE data from the different experimental systems described in this paper, titters determined at the National Institutes of Health are reported for all experiments except those involving infection with *N. brasiliensis*. 

**PGF2.** Suspensions of cells prepared from spleens of mice injected with LP-BM5 MuLV or with GaM8 were stimulated in vitro with LPS (20 μg/ml E. coli 0127-B8; L-4516; Sigma Chemical Co., St. Louis, MO). Culture supernatants harvested after 24 h were analyzed for PGF2 by radiolimunnoassay as described previously (24).

**RNA Purification and cDNA Synthesis.** Mouse spleen samples (40 mg) were stored at −70°C in RNazol (TEL-Test, Friendswood, TX) until further processing. RNA was extracted according to the manufacturer's directions. 1 μg RNA was reverse transcribed using MuLV reverse transcriptase (Promega Corp., Madison, WI) according to the recommendations of the manufacturer. The cDNA solution was diluted to 200 μl and 10 μl was used for specific amplification by PCR.

**PCR and Detection of the Amplified Products.** Primers for amplification and probes for detection of hypoxanthine phosphoribosyl transferase (HPRT), IL-2, -4, -6, -10, and -12, IFN-γ, pe, and ge transcripts have been described (8, 25). To amplify and detect the transcript of IL-13, we used the following primers:
using the ECL-3' oligolabeling and detection system and Hyperfilm-ECL (Amersham Corp., Arlington Heights, IL).

For quantification, we determined the intensity of hybridizing bands with a scanner (Hewlett Packard, Rockville, MD). To account for variability in starting cDNA concentration and integrity among the samples, cDNA from all samples was amplified with primers for HPRT. All values obtained for ge and pe were normalized to HPRT expression. For quantitative comparisons of the amounts of ge and pe RNA in different samples, we generated standard curves by serially diluting cDNA samples of B6 mice treated 7 d previously with GaM8. Amplification of ge for 33 cycles gave a linear correlation between amount of template cDNA and ge product whereas after 28 cycles of amplification pe product correlated directly with the logarithm of input cDNA (Fig. 1).

Results

IgE Production during the Course of MAIDS in IL-4−/− Mice. Development of MAIDS in disease-sensitive B6 mice is associated with enhanced expression of transcripts for the type 2 cytokines, IL-4 and IL-10, as well as with increased transcripts for the type 1 cytokines, IL-12, TNF-α, and IFN-γ (14, 27). In contrast, transcripts for IL-2 decrease with time after infection and progression of MAIDS (14, 27). To determine whether the balance of cytokines is

Table 1. B6 Mice Deficient for IL-4 (B6.IL-4−/−) Produce IgE during MAIDS

| Weeks after infection | IL-4 genotype | IgE (ng/ml) |
|----------------------|---------------|-------------|
|                      | +/+ (4)       | 79 ± 36     |
|                      | −/− (4)       | <0.5        |
| 3                    | +/+ (1)       | 33          |
|                      | −/− (3)       | 9 ± 9       |
| 6                    | +/+ (1)       | 448         |
|                      | −/− (3)       | 193 ± 84    |
| 9                    | +/+ (1)       | 222         |
|                      | −/− (3)       | 431 ± 16    |
| 14                   | +/+ (1)       | 32          |
|                      | −/− (4)       | 91 ± 43     |

B6.IL-4−/− and control IL-4+/+ littermates were injected intraperitoneally with 0.1 ml LP-BM5 MuLV virus pool. Mice were bled at the indicated time points, and serum IgE levels were determined by ELISA. Numbers in parentheses indicate the number of mice tested at each timepoint. Data for IgE levels indicate the mean ± 1 SE.
To determine whether comparable expression of IL-4 and wild-type IL-4 in IL-4-/- IL-4-deficient mice did not influence IL-4--independent IgE isotype switching in the mouse.

The results showed that IL-4-/- mice were capable of generating an IgE response after stimulation with GaM8, with serum levels peaking on day 7 at ~30 ng/ml and thus at 300-fold lower than wild-type mice which peaked at >10,000 ng/ml. These findings confirmed the suggestion that there is an IL-4-independent mechanism for induction of IgE switching in the mouse but indicated that in the GaM8 system this pathway is much less efficient than that operative in IL-4-competent mice. The IgG1 response in the B6 IL-4-/- mice reached 25% of the IgG1 response seen in wild-type controls, confirming earlier data showing that switching to IgG1 is not as dependent on IL-4 as switching to IgE.

Table 2. IgE and IgG1 Production after In Vivo GaM8 Immunization

| Days after immunization | Mice | IgE (ng/ml) | IgG1 (μg/ml) |
|-------------------------|------|-------------|-------------|
| 0                       | IL-4+/+ (8) | 33.5 ± 5.5 | 174 ± 59   |
|                         | IL-4+/+ (10) | <0.5       | 16 ± 4     |
|                         | BALB/c (8) | 67.5 ± 23  | 135 ± 64   |
| 5                       | IL-4+/+ (1) | 58         | 211        |
|                         | (3) | 5.3 ± 0.3   | 46 ± 12    |
|                         | BALB/c (1) | 159        | 76         |
| 7                       | IL-4+/+ (2) | 7,131 ± 2,869 | 957 ± 408 |
|                         | (4) | 26 ± 11.7   | 223 ± 80   |
|                         | BALB/c (2) | 7,814 ± 2,186 | 14,640 ± 3,040 |
| 8                       | IL-4+/+ (2) | >10,000    | 11,910     |
|                         | (3) | 22.7 ± 11.4 | 6,303 ± 4,593 |
|                         | BALB/c (2) | 6,436 ± 3,565 | ND         |
| 10                      | IL-4+/+ (2) | >10,000    | >25,000    |
|                         | (3) | 15.5 ± 3.5  | 5,168 ± 2,691 |
|                         | BALB/c (2) | 1,031 ± 1  | 24,325 ± 675 |

B6.IL-4-/-129, control IL-4+/+ littermates, and BALB/c mice were injected subcutaneously with 200 μl of GaM8 antiserum. Animals were killed on the indicated days, and serum IgE and IgG1 levels were determined by ELISA. Numbers in parentheses indicate the number of mice in each group. Data for IgE and IgG1 levels indicate the mean ± 1 SE.

A crucial determinant of sensitivity to MAIDS, we have examined the course of disease in mice unable to produce specific cytokines as the result of gene knockouts. We reported previously that IL-4 is not required for development of MAIDS, as B6 IL-4-/- mice were indistinguishable from controls for induction and progression of disease (28).

As part of this study, we examined the serum Ig isotypes in B6.IL-4-/-129 and normal mice during the first 12 wk after infection. Most noteworthy was the finding that B6.IL-4-/- mice had comparably high levels of serum IgE at wk 9 after infection (346 ± 194 and 817 ± 236 ng/ml, respectively). This finding runs against the current understanding of cytokine involvement in isotype switching in the mouse, which holds that switching to IgE is strictly dependent on IL-4. To determine whether these results were influenced by residual 129 genes in the B6.IL-4-/-129 congenic mice that may affect penetrance of the IgE-- dependent IgE switching in the mouse.

IgE Production after Treatment of IL-4-/- Mice with GaM8. To determine whether comparable expression of IgE in IL-4-/- and wild-type mice is a situation unique to MAIDS, we examined other in vivo systems associated with efficient induction of IgE. Treatment of mice with GaM8 stimulates polyclonal B cell activation and secretion of large amounts of IgE and IgG1 (30, 31). The increase in serum IgE has been shown to be dependent on the presence of IL-4, as treatment with anti-IL-4 mAb at the time of GaM8 administration almost completely ablates IgE secretion without affecting IgG1 production (6, 9). Although previous studies of (B6 × 129/SvJ)F1 IL-4-/- mice treated with GaM8 showed that serum IgE levels remained below the limits of detection (<15 ng/ml) (12), we chose to reexamine this response using B6.IL-4-/-129 mice.

IL-4-/- mice, control littermates, and BALB/c mice were injected subcutaneously with GaM8 antiserum, and serum IgE and IgG1 levels were followed for 10 d (Table 2). The results showed that IL-4-/- mice were capable of generating an IgE response after stimulation with GaM8, with serum levels peaking on day 7 at ~30 ng/ml and thus at 300-fold lower than wild-type mice which peaked at >10,000 ng/ml. These findings confirmed the suggestion that there is an IL-4-independent mechanism for induction of IgE switching in the mouse but indicated that in the GaM8 system this pathway is much less efficient than that operative in IL-4-competent mice. The IgG1 response in the B6 IL-4-/- mice reached 25% of the IgG1 response seen in wild-type controls, confirming earlier data showing that switching to IgG1 is not as dependent on IL-4 as switching to IgE.

ge and pe RNA Expression after GaM8 Treatment of IL-4-/- Mice. IL-4-dependent IgE isotype switching is characterized by induction of ge followed by pe RNA expression. We therefore wanted to determine whether the reduced serum IgE levels in IL-4-/- mice treated with GaM8 were due to impaired induction of ge and/or pe transcripts. Semi quantitative reverse transcription (RT)-PCR studies of RNA from spleens of treated mice revealed that IL-4-/- mice expressed only slightly reduced levels of ge transcripts compared with their controls (Fig. 2). The induction of pe transcripts, however, was significantly impaired in IL-4-/- mice (Fig. 2), thus explaining the reduced serum IgE levels found in these animals. Quantification of the RT-PCR transcripts showed that while levels of ge transcripts in IL-4-deficient mice reached 25 and 50% of the levels found in B6 control mice at days 5 and 7, respectively, levels of pe transcripts in IL-4-deficient mice were undetectable at day 5 and were 200-fold lower than in B6 mice on day 7. These findings indicate that although induction of ge transcripts can occur quite efficiently in the absence of IL-4, switch recombination and translation of pe transcripts are more IL-4 dependent than induction of ge transcripts.

Contact-mediated activation of B cells through CD40-CD40L interaction has been shown to induce ge transcripts in resting B cells in the absence of IL-4 in vitro (33). GaM8 immunization leads to activation of anti-goat Ig-specific T cells, which express CD40L and interact with the antigen-expressing B cells (Finkelman, F.D., unpublished observa-
Cytokine Gene Expression in B6.IL-4-/- Mice Injected with GaMß Antiserum. In vivo immunization with GaMß antiserum induces a specific and highly reproducible pattern of cytokine gene expression during the course of the primary immune response (25). We asked whether this expression pattern, particularly IFN-γ expression, might be altered in IL-4-/- mice. IFN-γ is known to inhibit IgE switching, and it has been suggested to downregulate ge transcripts (34, 35), although it does not suppress IgE secretion stimulated by anti-CD40 plus IL-4 (36). RT-PCR was used to follow the expression of IFN-γ, as well as IL-2, -4, -6, -10, and -13, at 5, 7, and 10 d after immunization (Fig. 2, and data not shown). We found no significant differences in the kinetics or levels of expression of these cytokines after treatment with GaMß, although B6 mice (IL-4-/- and controls) had slightly increased IFN-γ and decreased IL-13 levels compared with BALB/c mice. Nonproductive IL-4 transcripts characteristic of mice bearing the disrupted 129 IL-4 gene were constant throughout this time frame (data not shown).

Evaluation of the IL-4Rα chain in IL-4-Independent Induction of ge and pe RNA Expression and IgE Production in B6.IL-4-/- Mice Treated with GaMß Antiserum. The studies of mice treated with GaMß antiserum confirmed that there is an IL-4-independent mechanism for IgE switching in the mouse. It is conceivable, however, that the IL-4-independent and -dependent pathways of IgE switching share components of a common signal transduction pathway. It has been shown that IL-13 induces human B cells to switch to IgE (37). Furthermore, there is in vitro evidence in the mouse that IL-13 binds to and signals through an IL-4Rα/IL-13Rα heterodimer (38). To assess the possible role of the IL-4Rα chain in the IL-4-independent induction of IgE, B6.IL-4-/- mice, control littersmates, and BALB/c mice were treated with a combination of anti-IL-4 and anti-IL-4Rα mAb during stimulation with GaMß. Treatment of IL-4-deficient mice with the combination of IL-4 antagonists did not inhibit induction of ge and pe transcripts or the production of serum IgE (Fig. 3, and data not shown). Day 7 serum IgE levels in controls treated with anti-IL-4 and anti-IL-4Rα were in the range of pretreatment levels, indicating that the combination of IL-4 antagonists efficiently blocked IL-4-dependent IgE induction due to GaMß treatment (Fig. 3).

Studies of Switch Recombination at the ε Locus in IL-4-/- Mice. Expression of isotypes other than IgM and IgD is achieved by recombination events involving switch regions that are present upstream of each Cν, except C6. This recombination deletes the intervening Cν genes from the

Figure 2. Semiquantitative RT-PCR analysis of ge, pe, and cytokine expression after in vivo immunization with GaMß antiserum. (A) Unimmunized (control) and treated IL-4-deficient, (B) wild-type B6, and (C) BALB/c mice were killed on days 5, 7, and 10 after subcutaneous injection of 200 µl GaMß antiserum. RT-PCR, electrophoresis, Southern blotting, hybridization, and autoradiography were performed as described in Materials and Methods. The experiment shown is representative of three independent experiments.
chromosome. However, mechanisms other than deletional recombination may lead to expression of non-IgM isotypes. Alternative splicing of a long transcript which includes VDJ, Cμ, and Ce or trans-splicing between separate transcripts encoding VDJ and Ce have been proposed (39-43). Switch recombination to Ce can be analyzed by DC-PCR. Studies of genomic DNA (26, 44). DC-PCR analysis of splenic DNA isolated from IL-4-deficient mice infected with LP-BM5 for 3 wk or immunized for 5 and 7 d with GaM8 yielded the predicted 550-bp PCR fragment which hybridized with a specific Ce membrane region derived probe (Fig. 4). Thus, deletional switch recombination can occur in the absence of IL-4 and the Ce transcripts found in the IL-4-deficient mice are most likely to be derived from the switched locus.

Studies of Ce and RNA Expression and IgE Production after Infection of IL-4−/− Mice with N. brasiliensis. It was reported that (B6 × 129/Sv)F2 IL-4−/− mice failed to produce serum IgE (detection limit, 15 ng/ml) on infection with N. brasiliensis (11, 12). To examine whether strain background might influence IL-4-independent induction of IgE in this situation, we repeated the experiment with B6.IL-4−/− mice. Infection of B6 IL-4 knockout mice failed to induce any detectable serum IgE levels in a primary response or on reinfection (detection limit, 25 ng/ml), thus confirming earlier studies (11, 12). However, molecular studies of these mice revealed induction of Ce transcripts in spleen and mesenteric lymph node which peaked at day 7, while switched Ce transcripts levels were below the level of detection in spleen and barely detectable in mesenteric lymph node at day 11, when they peaked in the controls (Fig. 5).

To determine whether the failure to progress from Ce transcriptional activation to secretion of IgE in N. brasiliensis–infected, IL-4–deficient mice is modulated by IFN-γ,
Table 3. PGE₂ Production during MAIDS and after Immunization with GaM8

| Mice injected with | Time after injection | Mice | PGE₂ production after stimulation with |
|--------------------|----------------------|------|----------------------------------------|
|                    |                      |      | Medium | LPS |
|                    |                      |      | ng/ml  | ng/ml |
| BM5                | 0 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 1.2 ± 0.7 | 0.78 ± 0.3 |
| BM5                | 3 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 2.3 ± 1.7 | 1.76 ± 1 |
| BM5                | 6 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 0.7 ± 0.2 | 161.2 ± 125.3 |
| BM5                | 9 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 0.3 | 33.2 |
| BM5                | 6 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 2.8 ± 1.1 | 37.3 ± 12.1 |
| BM5                | 9 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 0.2 | 150 |
| BM5                | 9 wk                 | B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~
|                    |                      |      | 0.7 ± 0.3 | 66.24 ± 33.9 |
| GaM8               | 5 d                  | C57BL/6 | (2) | 0.02 ± 0.02 | 0.86 ± 0.33 |
| GaM8               | 7 d                  | C57BL/6 | (2) | 0.04 ± 0.04 | 1.68 ± 0.29 |

B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~

Mice were treated with a neutralizing mAb to IFN-γ at the time of primary and secondary infections. Serum IgE was below the limits of detection at days 14 and 21 of the primary response, but two of four mice produced IgE (51 ng/ml to 243 ng/ml) at days 14 and 21 of the secondary response. This indicates that the IL-4-independent pathway for IgE switching is subject to regulation by IFN-γ and suggests that it shares common elements with the IL-4-dependent pathway.

PGE₂ Production during MAIDS and after GaM8 Treatment of B6.IL-4⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~

Discussion

Both the mode of B cell activation and stimulating cytokines play important roles in determining the profile of secreted Ig isotypes by influencing isotype switching (50). Thus, B cells activated by anti-IgD antibodies conjugated to high molecular weight dextran in the presence of IL-4 and IL-5 fail to switch to IgE, but switching to IgE is induced by LPS and IL-4. In addition, some B cell signals may be sufficient to target certain C19 genes for switch rearrangement in the absence of cytokines. For example, membranes from activated T cells were shown to induce germ-line gY1 transcripts (32), and Sf9 cells expressing CD40L induced gY1 and gE transcripts in resting splenic B cells (33).

The systems used in the current study to examine IgE switching in the absence of IL-4 differ markedly and are likely to include aspects of T cell-B cell interactions,
unique to each system, that affect IgE class switching. In spite of these differences, all stimuli investigated induced expression of \( \text{ge} \), indicating that IL-4 is not required for this phase of IgE switching in vivo. The latter steps of \( \text{pe} \) transcription and substantial IgE secretion were fully independent of IL-4 only in mice with MAIDS. Thus, \( \text{pe} \) transcripts were much reduced in IL-4-/- mice injected with GaM8 antiserum and could barely be detected in mice infected with \( N. \) brasiliensis. In addition, only low levels of IgE could be detected in the sera of mice treated with GaM8 and none was detected in the sera of mice after a primary infection with \( N. \) brasiliensis, suggesting that induction and translation of \( \text{pe} \) are more IL-4-dependent than induction of \( \text{ge} \).

The DC-PCR analyses demonstrated that the \( \text{pe} \) transcripts detected in IL-4-deficient mice were generated, like those in wildtype animals, by deletional recombination. This excludes the possibility that IgE expression in IL-4-/- mice can explained by switching mechanisms fundamentally different than those used in wild-type mice.

Although the level of \( \text{ge} \) transcription has been shown to correlate with the level of subsequent IgE synthesis (8, 51), transcription per se might be insufficient to direct isotype switching (3, 52-54). Our in vivo data show that transcriptional activation of a germline \( \text{CH} \) locus does not necessarily lead to switch recombination and thus supports the notion that these two events are regulated separately. We cannot, however, exclude the possibility that the level of germline transcription itself regulates switch recombination.

The mechanisms responsible for differing levels of IL-4-independent expression of \( \text{ge} \), \( \text{pe} \), and IgE in each of the experimental conditions described are not known. There are extensive data in the literature showing that PGE2 promotes isotype switching to IgE in vitro (37, 46-48). The finding that splenocytes isolated from BM5-infected mice produce PGE2 in vitro upon stimulation with LPS, whereas splenocytes isolated from uninfected control mice or GaM8-immunized mice do not, indicates that the cells in the former case but not the latter have been primed for PGE2 production during MAIDS. Since induction of \( \text{ge} \) transcripts can occur in the absence of IL-4, it is conceivable that PGE2 acts in concert with factors responsible for \( \text{ge} \) transcription or with other factors that facilitate subsequent steps in IgE production, thus leading to the high IgE levels produced during MAIDS. Clearly more studies addressing the contribution of PGE2 to IgE switching need to be done.

It is of interest that the IL-4-independent pathway of IgE induction is subject to regulation by IFN-\( \gamma \), thus providing another indication that the IL-4-dependent and -independent pathways of IgE induction have some elements in common.

In MAIDS, B cells are the major target for infection and expression of the LP-BM5 defective virus (14) raising the possibility that switching to IgE might be activated downstream of the IL-4R by the unique Gag protein encoded by this virus. To test this, we infected the CH12.LX B cell lymphoma with the LP-BM5 defective virus and compared the parental and infected cells for expression of \( \text{ge} \), \( \text{pe} \), and IgE. Parental CH12.LX cells constitutively expressed very low levels of \( \text{ge} \), infection with LP-BM5 defective virus induced a 10-fold increase in \( \text{ge} \) levels (data not shown). The defective virus, however, did not induce any detectable \( \text{pe} \) or IgE protein (data not shown). This finding indicates that expression of the defective virus in B cells can augment \( \text{ge} \) expression but that the subsequent steps leading to IgE production require additional signals which are not provided for by the expression of the defective virus in the B cell.

A recent in vitro study showed that CD40-CD40L interaction can induce \( \text{ge} \) transcripts in mouse B cells in the absence of cytokines, albeit at very low levels (33). We are currently analyzing CD40L expression in our systems to evaluate its possible contribution to IL-4-independent IgE switching in vivo. Unfortunately, it will probably be impossible to evaluate the importance of this interaction in MAIDS, as recent studies showed that mice treated with anti-CD40L during the week after infection do not develop disease (55).

Human B cells can be induced to switch to IgE with either IL-4 or IL-13 (35, 56), and it has been shown that both cytokines signal through the IL-4R\( \alpha \) chain (38). Murine IL-13, however, failed to induce IgE switching in cultures of LPS-activated B cells and in cultures where purified B cells were costimulated by activated Th2 clones (37). It is conceivable that, in the mouse, another cytokine would signal through the IL-4R\( \alpha \) chain and induce IgE switching. Our studies of immunization with GaM8 in the presence of antibodies to IL-4 and IL-4R\( \alpha \) indicate that signaling through the IL-4R\( \alpha \) chain is not involved in IL-4-independent IgE induction in mice; however, an as-yet-unidentified signal might trigger this pathway at some point downstream of the receptor in IL-4-/- mice, thus leading to IgE switching.

While the studies reported here were in progress, IgE production in IL-4-deficient mice—although at substantially lower levels than in the controls—was reported for infection with \( Plasmodium \) chabaudi (57) and \( Leishmania \) major (58); however, no IgE was found in IL-4-deficient mice infected with the nematode \( Brugia \) malayi (59).

In summary, retroviral infection, infection with a parasite, and immunization with GaM8 result in IL-4-independent induction of germline \( \epsilon \) transcription in vivo, suggesting that the requirement for IL-4 in this step of isotype switching in vivo can be overcome dependent on the mode of B cell activation. Whether the signal for induction of germline transcription is delivered by a cytokine or by a cytokine independent has not been determined. The result of in vitro studies showing that CD40-CD40L interaction leads to IL-4-independent induction of germline \( \epsilon \) in resting splenic B cells (33) makes this cell contact-mediated signal a possible candidate for the stimulus to germline transcription in the absence of IL-4 in vivo.
B cell activation leading to induction of germline transcription in the absence of IL-4 in vivo does not necessarily result in switch recombination and IgE production. This may indicate that these steps are controlled by distinct mechanisms in vivo and suggest that they are more IL-4 dependent than induction of ge in itself. The systems used to examine the sequence of IgE switching and secretion are, however, much too different to allow any firm conclusions.

The authors gratefully acknowledge the expertise of Dr. T.N. Fredrickson in the histopathologic studies, the excellent technical assistance of Y. Kim and B. Seymour, and the skillful editorial assistance of B.R. Marshall in the preparation of the manuscript. We also thank M. Kopf (Basel Institute for Immunology) for providing the IL-4 targeting vector and L. Wahl (Laboratory of Immunopathology, National Institute of Dental Research, National Institutes of Health) for assistance in the PGE2 measurements.

This work was supported in part by contract N01-AI-45203 to Microbiological Associates, Inc., Bethesda, MD. DNAX Research Institute of Molecular and Cellular Biology is supported by Schering Plough Corporation. The work of R. Kühn, K. Rajewsky, and W. Müller is supported by the Bundesministerium für Forschung und Technologie through the Genzentrum Köln. N. Noben-Trauth is supported by the Max Planck Society.

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Received for publication 18 April 1996 and in revised form 7 August 1996.

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