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 (gϵ) transcripts with comparable efficiency in IL-$4^{-/-}$ mice and controls, but the levels of productive epsilon transcripts (pϵ) 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 gϵ 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 gϵ transcripts; however, pϵ 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 gϵ transcription and switch recombination to pϵ.

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 (C_H)[1] transcription, which is believed to target switch recombination to specific heavy chain isotypes (3, 4). In the mouse, IL-4 stimulates induction of gγ1 and germline epsilon (gϵ) 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 gϵ transcripts (6–8). Inhibition of increases in gϵ transcripts by

[1] *Abbreviations used in this paper:* C_H, heavy chain constant region; DC-PCR, digestion-circularization PCR; GaMδ, goat anti–mouse IgD; gϵ, germline epsilon; HPRT, hypoxanthine phosphoribosyl transferase; MAIDS, murine acquired immunodeficiency syndrome; MuLV, murine leukemia virus; pϵ, productive epsilon; RT-PCR, reverse-transcription PCR.

IL-4 antagonists is associated with failure to induce productive epsilon (pε) 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 GaMδ antiserum, and infection with *N. brasiliensis*. In this paper, we report that IL-4 is not required for induction of gε transcripts in either of these systems and that IL-4–deficient mice produced gε transcripts at levels comparable to those of controls after treatment with GaMδ; however, germline transcription of ε did not necessarily lead to switch recombination at this locus. Thus, even though IL-$4^{-/-}$ mice infected with *N. brasiliensis* produced gε transcripts, they generated only very low levels of pε transcripts and failed to switch to IgE. IL-$4^{-/-}$ mice treated with anti-IgD, however, did switch to pε 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-$4^{tm1Cgn129}$ = B6.IL-$4^{-/-129}$). Genetically pure B6 IL-$4^{-/-}$ mice (C57BL/6-IL-4^{tm1nnt} = B6.IL-$4^{-/B6}$; Noben-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), GaMδ (21), and rat anti–mouse IL-4R α chain (M1) (22) were as described previously. mAb GL117 (anti–*Escherichia coli* β-galactosidase) 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 GaMδ antiserum. Antibodies against IL-4 and IL-4Rα chain were injected intraperitoneally 24 h before treatment with GaMδ antiserum.

Infection of Mice with N. brasiliensis. Normal B6, B6.IL-$4^{-/-129}$, and B6.IL-$4^{+/-129}$ 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-ME 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 developing 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 DNAX 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, titers determined at the National Institutes of Health are reported for all experiments except those involving infection with *N. brasiliensis*.

PGE_2. Suspensions of cells prepared from spleens of mice injected with LP-BM5 MuLV or with GaMδ 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 PGE_2 by radioimmunoassay 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, gε, and pε transcripts have been described (8, 25). To amplify and detect the transcript of IL-13, we used the following primers:

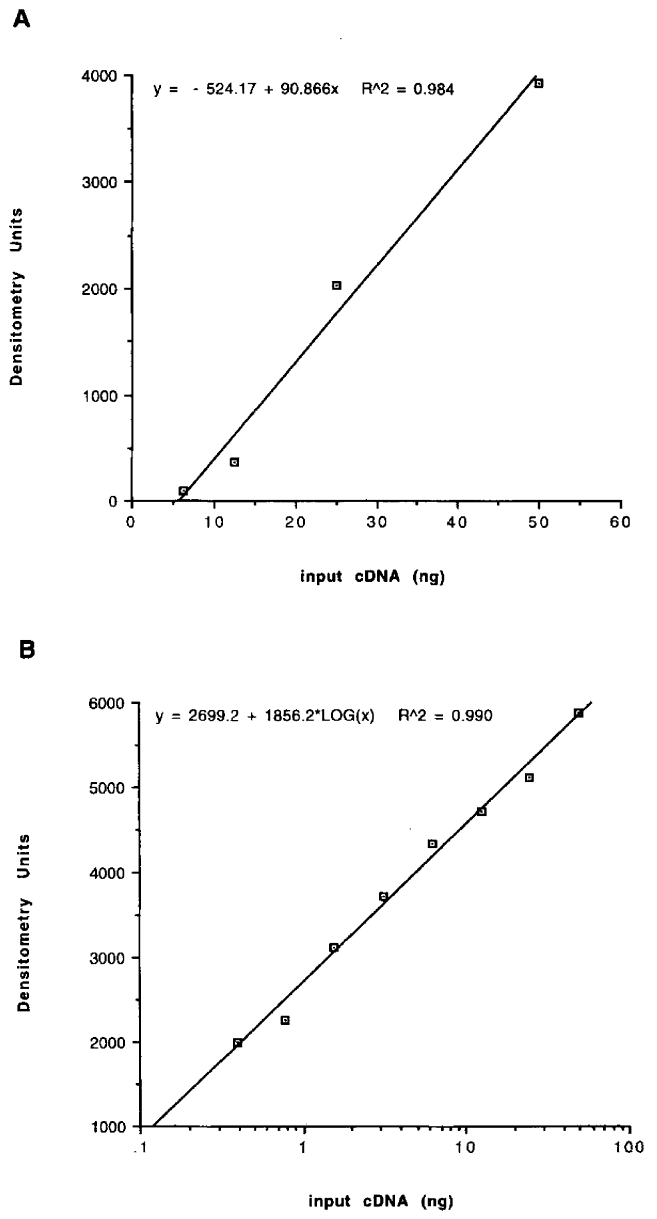

Figure 1. Video densitometric analysis of (*A*) gε and (*B*) pε RT-PCR signals as a function of the amount of input cDNA. cDNA obtained from spleens isolated from B6 mice immunized for 7 d with GaMδ antiserum was serially diluted 1/2 such that each dilution contained 50% of the previous dilution of cDNA. After amplification for 33 cycles (gε) and 28 cycles (pε) of RT-PCR, electrophoresis, Southern blotting, hybridization, and autoradiography were performed as described in Materials and Methods.

sense, 5′-ATGGCGCTCTGGGTGACT-3′; antisense, 5′-AATTGGAGATGTTGGTCAGGG-3′; and probe, 5′-GGAGTGTGGACCTGGCCG-3′. PCR was performed with 10 μl cDNA in 50-μl reaction volumes containing 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 0.2 mM for each of the dNTPs, 1.6 mM $MgCl_2$, 400 nM primers, and 1 U Taq DNA polymerase.

Digestion-circularization (DC)-PCR assays of genomic DNA from spleen cells were performed as described (26).

The PCR products were separated on 1.2% agarose gels, blotted onto a nylon membrane (Hybond-N^+, Amersham International, Birmingham, UK), and hybridized with a fluorescein-labeled probe 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 gε and pε were normalized to HPRT expression. For quantitative comparisons of the amounts of gε and pε RNA in different samples we generated standard curves by serially diluting cDNA samples of B6 mice treated 7 d previously with GaMδ. Amplification of gε for 33 cycles gave a linear correlation between amount of template cDNA and gε product whereas after 28 cycles of amplification pε 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$^{-/-B6}$) Produce IgE during MAIDS*

Weeks after infection	IL-4 genotype	IgE
		ng/ml
Experiment 1		
0	+/+ (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
Experiment 2		
4	+/+ (3)	294 ± 47
	−/− (4)	173 ± 72
8	+/+ (3)	1,578 ± 47
	−/− (3)	751 ± 299
12	+/+ (3)	711 ± 112
	−/− (2)	464 ± 272

B6.IL-4$^{-/-B6}$ 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.

Table 2. *IgE and IgG1 Production after In Vivo GaMδ Immunization*

Days after immunization	Mice		IgE	IgG1
			ng/ml	*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
	IL-4$^{-/-}$	(3)	5.3 ± 0.3	46 ± 12
	BALB/c	(1)	159	76
7	IL-4$^{+/+}$	(2)	7,131 ± 2,869	957 ± 408
	IL-4$^{-/-}$	(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
	IL-4$^{-/-}$	(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
	IL-4$^{-/-}$	(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 BALBc mice were injected subcutaneously with 200 μl of GaMδ 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$^{-/-129}$ and wild-type mice had comparably high levels of serum IgE at 9 wk 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 IL-4$^{-/-}$ genotype in mice infected with LP-BM5 MuLV (28, 29), the study was repeated in IL-4$^{-/-}$ mice generated with B6 ES cells, B6.IL-4$^{-/-B6}$ (Table 1). Again, comparable levels of IgE were detected in the sera of IL-4–deficient and wild-type mice, thus indicating that residual 129 genes in the B6.IL-4$^{-/-129}$ mice did not influence IL-4–independent IgE switching in the mouse.

IgE Production after Treatment of IL-4$^{-/-}$ Mice with GaMδ. 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 GaMδ 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 GaMd administration almost completely ablates IgE secretion without affecting IgG1 production (6, 9). Although previous studies of (B6 × 129/SvJ)F$_2$ IL-4$^{-/-}$ mice treated with GaMδ 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 GaMδ 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 GaMδ, with serum levels peaking on day 7 at ~30 ng/ml and thus at least 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 GaMδ 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 (10–12, 32).

gε and pε RNA Expression after GaMδ Treatment of IL-4$^{-/-}$ Mice. IL-4–dependent IgE isotype switching is characterized by induction of gε followed by pε RNA expression. We therefore wanted to determine whether the reduced serum IgE levels in IL-4$^{-/-}$ mice treated with GaMδ were due to impaired induction of gε and/or pε transcripts. Semiquantitative reverse transcription (RT)-PCR studies of RNA from spleens of treated mice revealed that IL-4$^{-/-}$ mice expressed only slightly reduced levels of gε transcripts compared with their controls (Fig. 2). The induction of pε 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 gε 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 pε 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 gε transcripts can occur quite efficiently in the absence of IL-4, switch recombination and translation of pε transcripts are more IL-4 dependent than induction of gε transcripts.

Contact-mediated activation of B cells through CD40-CD40L interaction has been shown to induce gε transcripts in resting B cells in the absence of IL-4 in vitro (33). GaMδ 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-

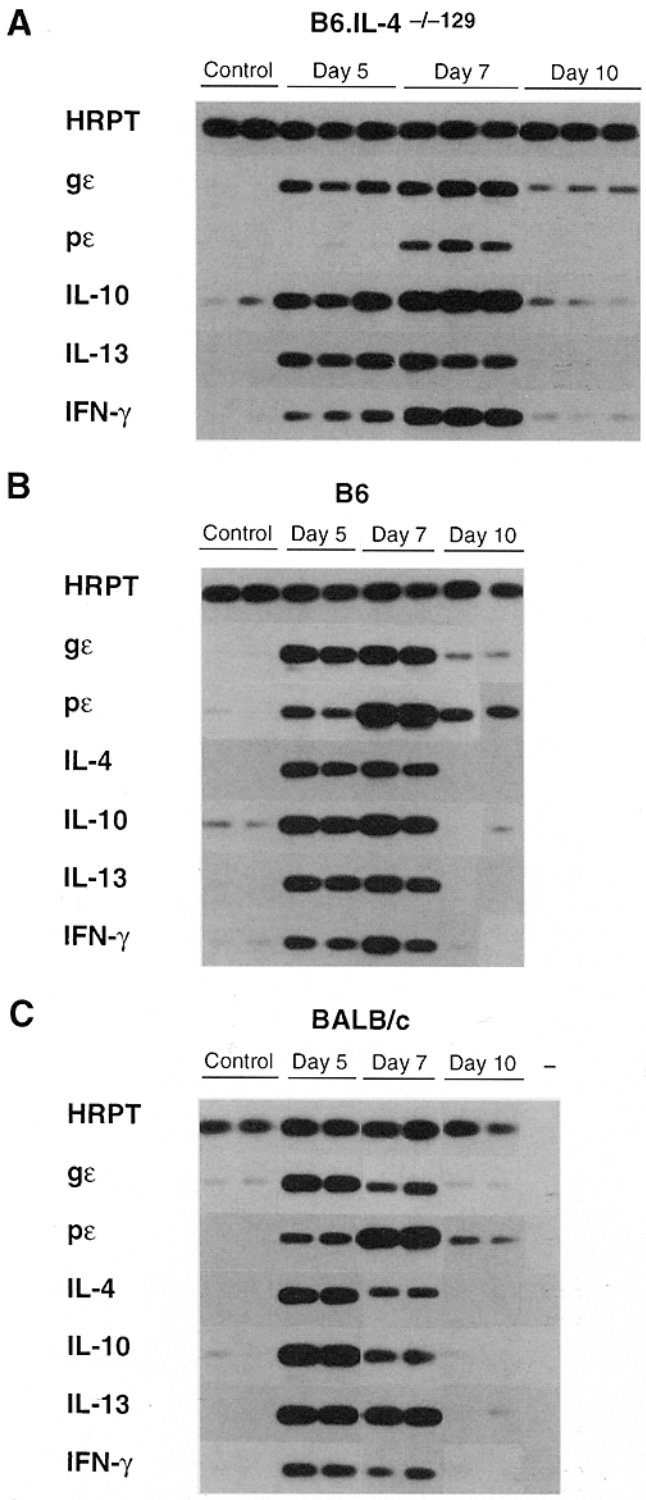

Figure 2. Semiquantitative RT-PCR analysis of gε, pε, and cytokine expression after in vivo immunization with GaMδ antiserum. (*A*) Uninoculated (*control*) and treated IL-4–deficient, (*B*) wild-type B6, and (*C*) BALB/c mice were killed on days 5, 7, and 10 after subcutaneous injection of 200 μl GaMδ 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.

tion). This interaction could account for the relatively high induction of gε levels seen in the IL-$4^{-/-}$ mice after injection of GaMδ. Our data suggest, however, that the rate-limiting step for IgE synthesis is not the induction of gε transcripts but rather switch recombination resulting in pε transcripts and subsequent translation of pε transcripts leading to IgE secretion and that IL-4 is involved in the regulation of these steps.

Cytokine Gene Expression in B6.IL-$4^{-/-}$ Mice Injected with GaMδ Antiserum. In vivo immunization with GaMδ antibody 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 do so by downregulating gε 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 GaMδ, 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-4Independent Induction of gε and pε RNA Expression and IgE Production in B6.IL-$4^{-/-}$ Mice Treated with GaMδ Antiserum. The studies of mice treated with GaMδ 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-$4^{-/-129}$ mice, control littermates, and BALB/c mice were treated with a combination of anti-IL-4 and anti-IL-4Rα mAb during stimulation with GaMδ. Treatment of IL-4–deficient mice with the combination of IL-4 antagonists did not inhibit induction of gε and pε 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 GaMδ 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 C_H, except Cδ. This recombination deletes the intervening C_H genes from the

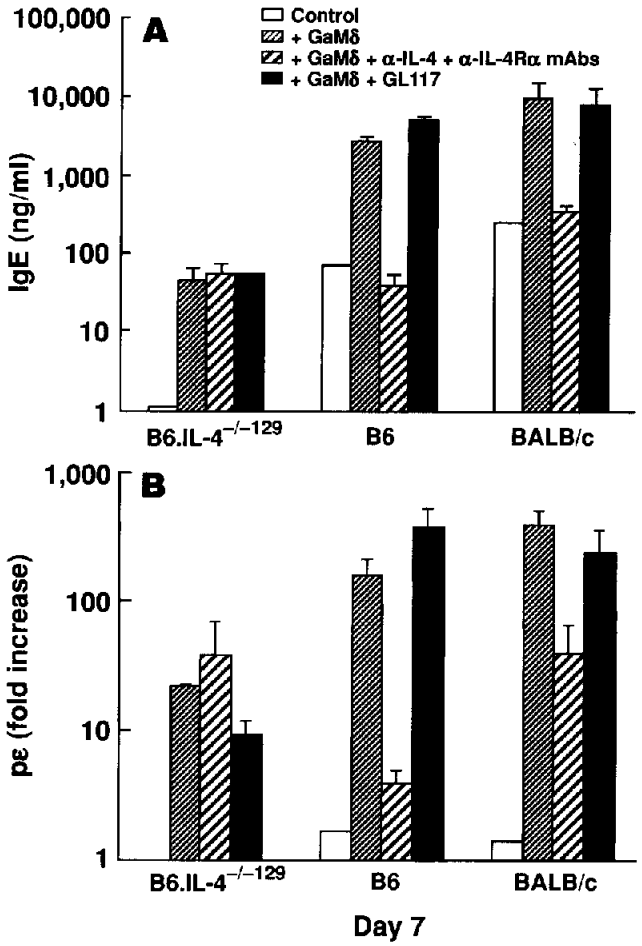

Figure 3. IL-4Rα dependence of pε RNA expression and IgE production in GaMδ-treated B6.IL-4 $^{-/-129}$, B6, and BALB/c mice. Mice (three mice per group) were injected intraperitoneally with 4 mg of neutralizing anti-IL-4 mAb (11B11) and 5 mg of IL-4R-blocking mAb (M1) or with isotype-matched control mAb (GL117). 24 h later the animals were injected subcutaneously with 200 μl of GaMδ antiserum. (*A*) Animals were killed at day 7 and serum IgE levels were determined by ELISA. (*B*) Splenic levels of pε mRNA were quantitated by RT-PCR and Southern blotting. Arithmetic means and standard errors of fold increases over unstimulated levels are shown.

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 Cε or *trans*-splicing between separate transcripts encoding VDJ and Cε have been proposed (39–43). Switch recombination to Sε 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 GaMδ yielded the predicted 550-bp PCR fragment which hybridized with a specific Cε membrane region derived probe (Fig. 4). Thus, deletional switch recombination can occur in the absence of IL-4 and the pε transcripts found in the IL-4–deficient mice are most likely to be derived from the switched locus.

Studies of gε and RNA Expression and IgE Production after Infection of IL-4 $^{-/-}$ Mice with N. brasiliensis. It was reported

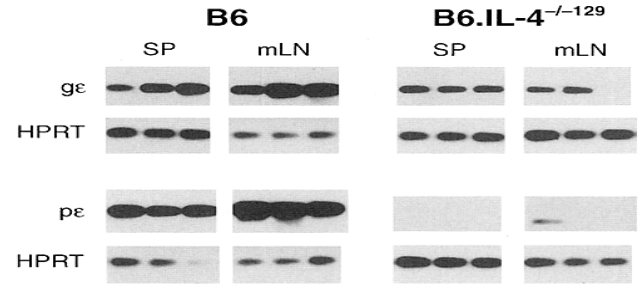

Figure 4. DC-PCR assay of genomic DNA after immunization with GaMδ or infection with LP-BM5. B6.IL-4$^{-/-129}$ and B6 mice were immunized with GaMδ and DNA was isolated from spleen cells at days 5 and 7 after immunization. B6.IL-4$^{-/-B6}$ and B6 mice were infected with LP-BM5 and DNA was isolated from spleen cells 3 wk later. DC-PCR, electrophoresis, Southern blotting, hybridization, and autoradiography were performed as described in Materials and Methods. Switch recombination was detected by the presence of a band hybridizing with an oligonucleotide from the membrane exon region of Cε.

that (B6 × 129/SvJ)F_2 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$^{-/-129}$ 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 gε transcripts in spleen and mesenteric lymph node which peaked at day 7, while switched pε 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 gε transcriptional activation to secretion of IgE in *N. brasiliensis*–infected, IL-4–deficient mice is modulated by IFN-γ,

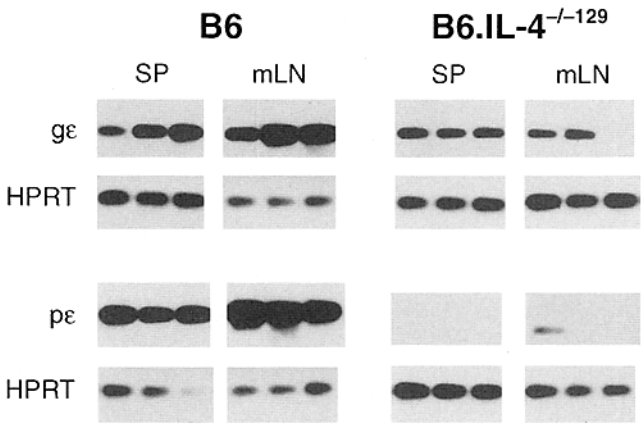

Figure 5. gε and pε expression in *N. brasiliensis*–infected B6.IL-4$^{-/-129}$ and B6 mice. Mice (three per group) were infected with *N. brasiliensis*. Mice were killed on days 5, 7, and 11 and spleen and mesenteric lymph nodes harvested for RNA preparation. RT-PCR, electrophoresis, Southern blotting, hybridization, and autoradiography were performed as described in Materials and Methods. Shown are day 7 gε transcript levels and day 11 pε transcript levels, which represent their peak levels.

Table 3. *PGE_2 Production during MAIDS and after Immunization with GaMδ*

Mice injected with	Time after injection	Mice		PGE_2 production after stimulation with: Medium	PGE_2 production after stimulation with: LPS
				ng/ml	*ng/ml*
BM5	0 wk	B6.IL-$4^{+/+B6}$	(4)	1.2 ± 0.7	0.78 ± 0.3
		B6.IL-$4^{-/-B6}$	(2)	2.3 ± 1.7	1.76 ± 1
BM5	3 wk	B6.IL-$4^{+/+B6}$	(1)	0.03	78.7
		B6.IL-$4^{-/-B6}$	(2)	0.7 ± 0.2	161.2 ± 125.3
BM5	6 wk	B6.IL-$4^{+/+B6}$	(1)	0.3	33.2
		B6.IL-$4^{-/-B6}$	(3)	2.8 ± 1.1	37.3 ± 12.1
BM5	9 wk	B6.IL-$4^{+/+B6}$	(1)	0.2	150
		B6.IL-$4^{-/-B6}$	(3)	0.7 ± 0.3	66.24 ± 33.9
GaMδ	5 d	C57BL/6	(2)	0.02 ± 0.02	0.86 ± 0.33
		B6.IL-$4^{-/-129}$	(2)	0.04 ± 0.04	1.68 ± 0.29
GaMδ	7 d	C57BL/6	(2)	<0.01	2.81 ± 0.14
		B6.IL-$4^{-/-129}$	(2)	0.03 ± 0.03	4.76 ± 0.42

B6.IL-$4^{-/-B6}$ and control IL-$4^{+/+}$ littermates were injected intraperitoneally with 0.1 ml LP-BM5 MuLV virus pool. Animals were killed at the indicated time points and the spleens isolated. B6.IL-$4^{-/-129}$ and B6 mice were injected subcutaneously with 200 μl of GaMδ antiserum. Animals were killed on the indicated days, and the spleens isolated. Suspensions of cells prepared from spleen of mice injected with LP-BM5 MuLV or with GaMδ were stimulated in vitro with LPS. Culture supernatants harvested after 24 h were analyzed for PGE_2 by radioimmunoassay as described in Materials and Methods. Numbers for PGE_2 indicate the mean ± 1 SE.

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.

PGE_2 Production during MAIDS and after GaMδ Treatment of B6.IL-$4^{-/-}$ and Control Mice. IgE induction in IL-4–deficient mice after GaMδ immunization and *N. brasiliensis* infection was very low or even absent, in sharp contrast to the elevated IgE levels seen during the course of MAIDS. Increases in cell proliferation or viability, an increase in the percentage of cells undergoing IgE class switching, an increase in the average amount of IgE secreted per cell, or a combination of the above could result in higher IgE levels seen during MAIDS. We therefore asked what additional mechanisms might contribute to IgE switching during MAIDS.

PGE_2 and other agents that increase intracellular cAMP have been shown to synergize with IL-4 and LPS to induce IgE and IgG1 production in normal mice (45–48). They do so by increasing the number of splenic B cells secreting IgE, by promoting class switching of IgM^+ B cells, and by synergizing with IL-4 to induce gε transcripts. The possibility that expression of PGE_2 might be elevated in MAIDS is suggested by the observation that the course of disease is delayed in mice treated with meclofenamic acid, an inhibitor of prostaglandin synthase (49). To address this possibility, spleen cells from mice with MAIDS were stimulated with LPS and the supernatants tested for PGE_2 (Table 3). These studies revealed that significant levels of PGE_2 were produced by stimulated spleen cells of both IL-$4^{-/-}$ and wild-type mice. In contrast, only low levels of PGE_2 were produced by spleen cells of mice treated with GaMδ. These results suggest that enhanced expression of prostaglandins may contribute to elevated IgE expression in IL-$4^{-/-}$ mice with MAIDS.

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 C_H genes for switch rearrangement in the absence of cytokines. For example, membranes from activated T cells were shown to induce germline gγ1 transcripts (32), and Sf9 cells expressing CD40L induced gγ1 and gε 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 gε, indicating that IL-4 is not required for this phase of IgE switching in vivo. The latter steps of pε transcription and substantial IgE secretion were fully independent of IL-4 only in mice with MAIDS. Thus, pε transcripts were much reduced in IL-4$^{-/-}$ mice injected with GaMδ 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 GaMδ and none was detected in the sera of mice after a primary infection with *N. brasiliensis*, suggesting that induction and translation of pε are more IL-4 dependent than induction of gε.

The DC-PCR analyses demonstrated that the pε 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 gε 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 C_H 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 gε, pε, and IgE in each of the experimental conditions described are not known. There are extensive data in the literature showing that PGE_2 promotes isotype switching to IgE in vitro (37, 46–48). The finding that splenocytes isolated from BM5-infected mice produce PGE_2 in vitro upon stimulation with LPS, whereas splenocytes isolated from uninfected control mice or GaMδ-immunized mice do not, indicates that the cells in the former case but not the latter have been primed for PGE_2 production in vivo. This suggests that PGE_2 may also contribute to the high IgE levels produced during MAIDS. Since induction of gε transcripts can occur in the absence of IL-4, it is conceivable that PGE_2 acts in concert with factors responsible for gε transcription or with other factors that facilitate subsequent steps in IgE production, thus leading to the high levels of IgE seen during MAIDS. Clearly more studies addressing the contribution of PGE_2 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-γ, 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 gε, pε, and IgE. Parental CH12.LX cells constitutively expressed very low levels of gε, infection with LP-BM5 defective virus induced a 10-fold increase in gε levels (data not shown). The defective virus, however, did not induce any detectable pε or IgE protein (data not shown). This finding indicates that expression of the defective virus in B cells can augment gε 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 gε 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-4Rα 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-4Rα chain and induce IgE switching. Our studies of immunization with GaMδ in the presence of antibodies to IL-4 and IL-4Rα indicate that signaling through the IL-4Rα 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 GaMδ result in IL-4–independent induction of germline ε 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 is 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 ε 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 gε 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 PGE_2 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.

References

1. Armitage R.J., W.C. Fanslow, L. Strockbine, T.A. Sato, K.N. Clifford, B.M. Macduff, D.M. Anderson, S.D. Gimpel, T. Davis-Smith, C.R. Maliszewski et al. 1992. Molecular and biological characterization of a murine ligand for CD40. *Nature (Lond.).* 357:80–82.
2. Renshaw, B.R., W.C. Fanslow III, R.J. Armitage, K.A. Campbell, D. Liggitt, B. Wright, B.L. Davison, and C.R. Maliszewski. 1994. Humoral immune response in CD40 ligand-deficient mice. *J. Exp. Med.* 180:1889–1900.
3. Stavnezer, J., G. Radcliffe, Y.C. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson. 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA.* 85:7704–7708.
4. Rothman P., S. Lutzker, W. Cook, R. Coffman, and F.W. Alt. 1988. Mitogen plus interleukin-4 induction of cε transcripts in B lymphoid cells. *J. Exp. Med.* 163:2385–2389.
5. Coffman, R.L., D.A. Lebman, and P. Rothman. 1993. Mechanism and regulation of immunoglobulin isotype switching. *Adv. Immunol.* 54:229–270.
6. Finkelman, F.D., I.M. Katona, J.F. Urban, Jr., J. Holmes, J. Ohara, A.S. Tung, J.V. Sample, and W.E. Paul. 1988. IL-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* 141:2335–2341.
7. Katona, I.M., J.F. Urban, Jr., S.S. Kang, W.E. Paul, and F.D. Finkelman. 1991. IL-4 requirements for the generation of secondary in vivo IgE responses. *J. Immunol.* 146:4215–4221.
8. Thyphronitis, G., I.M. Katona, W.C. Gause, and F.D. Finkelman. 1993. Germline and productive Cε gene expression during in vivo IgE responses. *J. Immunol.* 151:4128–4136.
9. Finkelman, F.D., I.M. Katona, J.F. Urban, Jr., C.M. Snapper, J. Ohara, and W.E. Paul. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc. Natl. Acad. Sci. USA.* 83:9675–9678.
10. Finkelman, F.D., J.F. Urban, Jr., M.P. Beckmann, K.A. Schooley, J.M. Holmes, and I.M. Katona. 1991. Regulation of murine in vivo IgG and IgE responses by a monoclonal anti-IL-4 receptor antibody. *Int. Immunol.* 3:599–607.
11. Kühn, R., K. Rajewsky, and W. Müller. 1991. Generation and analysis of interleukin-4 deficient mice. *Science (Wash. DC).* 254:707–710.
12. Kopf, M., G.L. Gros, M. Bachmann, M.C. Lamers, H. Bluethmann, and G. Koehler. 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature (Lond.).* 362:245–248.
13. Morse, H.C., III, S.K. Chattopadhyay, M. Makino, T.N. Fredrickson, A.W. Hügin, and J.W. Hartley. 1992. Retrovirus-induced immunodeficiency in the mouse: MAIDS as a model for AIDS. *AIDS.* 6:607–621.
14. Morse, H.C., III, N. Giese, R. Morawetz, Y. Tang, R. Gazzinelli, W. K. Kim, S. Chattopadhyay, and J.W. Hartley. 1995. Cells and cytokines in the pathogenesis of MAIDS, a retrovirus-induced immunodeficiency syndrome of mice. *Springer Semin. Immunopathol.* 17:231–245.
15. Lederman, B., and K. Bürki. 1991. Establishment of a germline competent C57BL/6 embryonic stem cell line. *Exp. Cell Res.* 197:254–259.
16. Chattopadhyay, S.K., H.C. Morse III, M. Makino, S.K. Ruscetti, and J.W. Hartley. 1989. A defective virus is associated with induction of a murine retrovirus-induced immunodeficiency syndrome, MAIDS. *Proc. Natl. Acad. Sci. USA.* 86: 3862–3866.
17. Hartley, J.W., T.N. Fredrickson, R.A. Yetter, M. Makino, and H.C. Morse III. 1989. Retrovirus-induced murine ac-

quired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. *J. Virol.* 63:1223–1231.

18. Klinman, D.M., and H.C. Morse III. 1989. Characteristics of B cell proliferation and activation in murine AIDS. *J. Immunol.* 142:1144–1149.
19. Gazzinelli, R.T., M. Makino, S.K. Chattopadhyay, C.M. Snapper, A. Sher, A.W. Hügin, and H.C. Morse III. 1992. $CD4^+$ subset regulation in viral infection. Preferential activation of Th2 cells during progression of retrovirus-induced immunodeficiency in mice. *J. Immunol.* 148:182–188.
20. Ohara, J., and W.E. Paul. 1985. B cell stimulatory factor BSF-1: production of a monoclonal antibody and molecular characterization. *Nature (Lond.).* 315:333–336.
21. Finkelman, F.D., S.W. Kessler, J.F. Mushinski, and M. Potter. 1981. IgD secreting murine plasmacytomas: identification and characterization of two IgD myeloma proteins. *J. Immunol.* 126:680–687.
22. Beckman, M.P., K.A. Schooley, M. Gallis, T. van den Bos, D. Friend, A.R. Alpert, R. Raunio, K.S. Prickett, P.E. Paker, and L.S. Park. 1990. Monoclonal antibodies block murine IL-4 receptor function. *J. Immunol.* 144:4212–4217.
23. Rizzo, L.V., R.H. DeKruyff, and D.T. Umetsu. 1992. Generation of B cell memory and affinity maturation. Induction with Th1 and Th2 clones. *J. Immunol.* 148:3733–3739.
24. Wahl, L.M. 1982. *In* Manual of Macrophage Methodology. H.B. Herscowitz, H.T. Holden, J.A. Bellanti, and A. Ghaffar, editors. Marcel Dekker Inc., New York. 423–429.
25. Svetic, A., F.D. Finkelman, Y.C. Jian, C.W. Dieffenbach, D.E. Scott, K.F. McCarthy, A.D. Steinberg, and W.C. Gause. 1991. Cytokine gene expression after in vivo primary immunization with goat antibody to mouse IgD. *J. Immunol.* 147:2391–2397.
26. Xu, L., and P. Rothman. 1994. IFN-γ represses ϵ germline transcription and subsequently down-regulates switch recombination to ϵ. *Int. Immunol.* 6:515–521.
27. Giese, N.A., R.T. Gazzinelli, J. Actor, R.A. Morawetz, M. Sarzotti, and H.C. Morse III. 1996. Retroviral-elicited interleukin-12 and TNF-α as inducers of IFN-γ-mediated pathology in MAIDS. *Immunology.* 87:467–474.
28. Morawetz, R., T.M. Doherty, N. Giese, J. Hartley, W. Müller, R. Kühn, K. Rajewsky, R. Coffman, and H.C. Morse, III. 1994. Resistance to murine acquired immunodeficiency syndrome (MAIDS). *Science (Wash. DC).* 265:264–267.
29. Kanagawa, O., B.A. Vaupel, S. Gayama, G. Koehler, and M. Kopf. 1993. Resistance of mice deficient in IL-4 to retrovirus-induced immunodeficiency syndrome (MAIDS). *Science (Wash. DC).* 262:240–242.
30. Finkelman, F.D., I. Scher, J.J. Mond, S. Kessler, J.T. Kung, and E.S. Metcalf. 1982. Polyclonal activation of the murine immune system by an antibody to IgD. II. Generation of polyclonal antibody production and cells with surface IgG. *J. Immunol.* 129:638–646.
31. Finkelman, F.D., C.M. Snapper, J.D. Mountz, and I.M. Katona. 1987. Polyclonal activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction of a polyclonal IgE response. *J. Immunol.* 138:2826–2830.
32. Schultz, C.L., P. Rothman, R. Kühn, M. Kehry, W. Mueller, K. Rajewsky, F. Alt, and R.L. Coffman. 1992. T helper membranes promote IL-4 independent expression of germline Cγ1 transcripts in B cells. *J. Immunol.* 149:60–64.
33. Warren, W.D., and M.T. Berton. 1995. Induction of germline γ1 and ϵ Ig gene expression in murine B cells. *J. Immunol.* 155:5637–5646.
34. Thyphronitis, G., J. Banchereau, C. Heusser, G. Tsokos, A. Levine, and F. Finkelman. 1991. Kinetics of IL-4 induction and interferon-γ inhibition of IgE secretion by Epstein-Barr virus-infected human peripheral blood B cells. *Cell. Immunol.* 133:408–419.
35. Punnonen, J., B.G. Cocks, and J.E. de Vries. 1995. IL-4 induces germ-line IgE heavy chain gene transcription in human fetal pre-B cells. *J. Immunol.* 155:4248–4254.
36. Gascan, H., J.F. Gauchat, G. Aversa, P. van Vlasselaer, and J.E. de Vries. 1991. Anti-CD40 monoclonal antibodies or $CD4^+$ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. *J. Immunol.* 147:8–13.
37. Zurawski, G., and J.E. de Vries. 1994. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today.* 15:19–26.
38. Hilton, D.J., J.G. Zhang, D. Metcalf, W.S. Alexander, N.A. Nicola, and T.A. Willson. 1996. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc. Natl. Acad. Sci. USA.* 93:497–501.
39. Perlmutter, A.P., and W. Gilbert. 1984. Antibodies of the secondary response can be expressed without switch recombination in normal mouse B cells. *Proc. Natl. Acad. Sci. USA.* 81:7189–7193.
40. Chen, Y.W., C. Word, V. Dev, J.W. Uhr, E.S. Vitetta, and P.W. Tucker. 1986. Double isotype production by a neoplastic B cell line. *J. Exp. Med.* 164:562–579.
41. MacKenzie, T., and H.M. Dosch. 1989. Clonal and molecular characteristics of the human IgE-committed B cell subset. *J. Exp. Med.* 169:407–430.
42. Chan, M.A., S.H. Benedict, H.M. Dosch, M.F. Hui, and L.D. Stein. 1990. Expression of IgE from a nonrearranged ϵ locus in cloned B-lymphoblastoid cells that also express IgM. *J. Immunol.* 144:3563–3568.
43. Nolan-Willard, M., M.T. Berton, and P. Tucker. 1992. Coexpression of μ and γ1 heavy chains can occur by a discontinuous transcription mechanism from the same unrearranged chromosome. *Proc. Natl. Acad. Sci. USA.* 89:1234–1238.
44. Chu, C.C., W.E. Paul, and E.E. Max. 1992. Quantitation of immunoglobulin μ-γ1 heavy chain switch region recombination by a digestion-circularization polymerase chain reaction method. *Proc. Natl. Acad. Sci. USA.* 89:6978–6982.
45. Roper, R.L., D.M. Brown, and R.P. Phipps. 1995. Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE. *J. Immunol.* 154:162–170.
46. Ohmori, H., M. Hikida, and T. Takai. 1990. Prostaglandin E_2 as a selective stimulator of antigen-specific IgE response in murine lymphocytes. *Eur. J. Immunol.* 20:2499–2503.
47. Roper, R.L., D.H. Conrad, D.M. Brown, G.L. Warner, and R.P. Phipps. 1990. Prostaglandin E_2 promotes IL-4-induced IgE and IgG1 synthesis. *J. Immunol.* 145:2644–2651.
48. Roper, R.L., and R.P. Phipps. 1992. Prostaglandin E_2 and cAMP inhibit B lymphocyte activation and simultaneously promote IgE and IgG1 synthesis. *J. Immunol.* 149:2984–2991.
49. Stadler, I., K.C. Chada, S. Nakeeb, C. Toumbis, J. Butsch, N. Mathur, F. Munschauer, A. Vladutiu, S.K. Satchidanand, and J.L. Ambrus. 1994. Pentoxifylline and meclofenamic acid treatment reduces clinical manifestations in a murine model of AIDS. *J. Pharm. Exp. Ther.* 268:10–13.
50. Snapper, C., and J.J. Mond. 1993. Towards a comprehensive view of immunoglobulin class switching. *Immunol. Today.* 14:

15–17.

51. Ichziki, T., W. Takahashi, and T. Watanabe. 1993. Regulation of the expression of human Cε germline transcripts. Identification of a novel IL-4 responsive element. *J. Immunol.* 150:5408–5417.
52. Gauchat, J.F., H. Gascan, R. de Waal Malefyt, and J.E. de Vries. 1992. Regulation of germ-line ε transcription and induction of ε switching in cloned EBV-transformed and malignant human B cell lines by cytokines and $CD4^+$ T cells. *J. Immunol.* 148:2291–2299.
53. Bottaro, A., R. Lansford, L. Xu, P. Rothman, and F.W. Alt. 1994. S region transcription per se promotes basal IgE class switch recombination but additional factors regulate the efficiency of the process. *EMBO (Eur. Mol. Biol. Organ.) J.* 13: 665–674.
54. Lorenz, M., S. Jung, and A. Radbruch. 1995. Switch transcripts in immunoglobulin class switching. *Science (Wash. DC).* 267:1825–1828.
55. Green, K.A., K.M. Crassi, J.D. Laman, A. Schoneveld, R.R. Strawbridge, T.M. Foy, R.J. Noelle, and W.R. Green. 1996. Antibody to the ligand for CD40 (gp39) inhibits murine AIDS-associated splenomegaly, hypergammaglobulinemia, and immunodeficiency in disease-susceptible C57BL/6 mice. *J. Virol.* 70:2569–2575.
56. Punnonen, J., G. Aversa, B.G. Cocks, A.N. McKenzie, S. Menon, G. Zurawski, R. de Waal Malefyt, and J.E. de Vries. 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc. Natl. Acad. Sci. USA.* 90:3730–3734.
57. von der Weid, T., M. Kopf, G. Koehler, and J. Langhorne. 1994. The immune response to *Plasmodium chabaudi* malaria in interleukin-4-deficient mice. *Eur. J. Immunol.* 24:2285–2293.
58. Noben-Trauth, N., P. Kropf, and I. Müller. 1996. Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. *Science (Wash. DC).* 271:987–990.
59. Lawrence, R.A., J.E. Allen, W.F. Gregory, M. Kopf, and R.M. Maizels. 1995. Infection of IL-4-deficient mice with the parasitic nematode *Brugia malayi* demonstrates that the host resistance is not dependent on a T helper 2–dominated immune response. *J. Immunol.* 154:5995–6001.