could also explain why a mIgM molecule that carries a Y → F mutation in its transmembrane part and presumably is less tightly associated with the Ig-α,Ig-β heterodimer than wild-type mIgM does not reach the antigen-presentation compartment (*16*).

B cells require T cell help for the production and affinity maturation of antibodies against protein antigens (*17*). Our finding that B cells with a tailless IgG2a-BCR cannot efficiently present antigen to T cells explains the reduction in IgG1 and IgE serum concentrations in mutant mice that express tailless IgG1-BCR and IgE-BCR, respectively (*18*). Activated memory B cells appear to require the cytoplasmic tails of mIg molecules and the efficient antigen presentation connected with this structure in order to expand and differentiate into antibody-producing plasma cells. Thus, the conserved COOH-terminal sequences of mIg molecules are finally assigned an important immunological function.

REFERENCES AND NOTES

1. M. R. Gold and A. L. DeFranco, *Adv. Immunol.* **55**, 221 (1994); C. M. Pleiman, D. D'Ambrosio, J. C. Cambier, *Immunol. Today* **15**, 393 (1994); M. S. Neuberger *et al.*, *Immunol. Rev.* **132**, 147 (1993); M. Reth, *Annu. Rev. Immunol.* **10**, 97 (1992).
2. K. J. Patel and M. S. Neuberger, *Cell* **74**, 939 (1993); A. Lanzavecchia, *Curr. Opin. Immunol.* **8**, 348 (1996).
3. A. R. Venkitaraman, G. T. Williams, P. Dariavach, M. S. Neuberger, *Nature* **352**, 777 (1991).
4. The expression vector pSVneoγ2amtl is a derivative of pSVγ2am. For the construction of this vector we used two overlapping oligonucleotides as a linker to introduce a Bgl II site and a stop codon after the sequence coding for the cytoplasmic amino acids KVK. Point mutations were introduced into the sequence coding for the γ2am tail by the polymerase chain reaction (PCR). As a template for PCR, we used the linearized pγ2am plasmid. The PCR products were cloned into pγ2am from which we generated the expression vectors pSV2neoγ2amY20L and pSV2neoγ2amY20L,M23L. The expression vectors for the chimeric scAgR were constructed from psc-cyt (R. Müller and M. Reth, in preparation), a derivative of the vector pHβAPr1neoFvCD3ζ (*9*). The psc-cyt vector encodes the V_H and V_L domains of the NIP-specific antibody B1-8, followed by a CD8α linker region and the CD3ζ transmembrane part, and carries a Bam HI cloning site after the CD3ζ sequence. Bam HI fragments of the wild-type or mutated cytoplasmic γ2am and mb-1 coding sequences were obtained by PCR and inserted into psc-cyt to yield the expression vectors pANPCD8γ2am, pANPCD8γ2amY20L, pANPCD8γ2amY20L,M23L, pANPCD8mb-1, and pANPCD8mb-1M1. The pANPCD8tl vector encodes a scAgR with a short cytoplasmic sequence of the five amino acids RRIDP. The vectors were linearized with Pvu I and introduced into K46 and K46λ12 cells by electroporation.
5. P. Weiser, C. Riesterer, M. Reth, *Eur. J. Immunol.* **24**, 665 (1994).
6. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; G, Gly; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; R, Arg; S, Ser; V, Val; X, any amino acid; and Y, Tyr.
7. Transfected cells were surface-biotinylated and lysed with 1% digitonin buffer as described (*5*). After a preclearing step with Sepharose beads, NIP-specific molecules were immunoprecipitated from the lysates with the use of NIP-Sepharose or Sepharose as control. After being washed three times with the 1% digitonin buffer, the NIP-Sepharose beads were boiled in reducing SDS sample buffer. The released proteins were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and electroblotted onto a nitrocellulose membrane. Biotinylated proteins were detected with Str-HRP and the ECL detection system (Amersham, Braunschweig, Germany).
8. Antigen presentation of K46-transfectants exposed to the antigen NIP-OVA was tested by the ability of the T cell hybridoma 3DO54.8 (which recognizes the OVA peptide 323-339 in the context of the I-A^d major histocompatibility complex class II molecule) to secrete interleukin 2 (IL-2). IL-2 production was quantitated in chromogenic proliferation assay (EZ4U, Biomedica, Vienna) using the IL-2–dependent cell line CTLL. The K46 transfectant and the 3DO54.8 line (4×10^4 cells each) were incubated in a 96-well plate for 24 hours in Dulbecco's modified Eagle's medium (DMEM) with different concentrations of NIP-OVA or OVA. The plates were then frozen at −80°C, and after thawing, the cell-free supernatant of each well was incubated for 24 hours with 3000 IL-2–starved CTLL cells. As high and low controls, CTLL cells were cultured with or without excess of exogenous IL-2. After addition of substrate solution and incubation for 3 to 5 hours, absorbance at 450 nm (OD_{450}) was measured on a Molecular Devices ELISA reader and analyzed with the program Softmax. The calibration of the assay was done by the formula (sample OD_{450} − low control OD_{450})/(high control OD_{450} − low control OD_{450}) and is presented as relative OD_{450}. Mean values and standard deviations from triplicates were then used to plot the dose-response curves. The statistical relevance of each mean value was verificated by use of Student's *t* test. Apart from transfectant K46λγ2amY20L, two or three independent clones of each transfectant were analyzed. B cells were propagated in RPMI 1640 medium containing 10% fetal calf serum (FCS) (Vitromex, Vilshofen, Germany) and G418 (0.5 mg/ml); the T cells were cultured in DMEM containing 10% FCS and, in case of the CTLL cells, 5% of supernatant of the IL-2–producing transfectant X63-IL-2.
9. T. Brocker, A. Peter, A. Traunecker, K. Karjalainen, *Eur. J. Immunol.* **23**, 1435 (1993).
10. A. L. M. Bothwell *et al.*, *Cell* **24**, 625 (1981).
11. C. Bonnerot *et al.*, *Immunity* **3**, 335 (1995).
12. H. Flaswinkel and M. Reth, *EMBO J.* **13**, 83 (1994).
13. K. M. Kim, G. Alber, P. Weiser, M. Reth, *Eur. J. Immunol.* **23**, 911 (1993).
14. J. L. Goldstein, M. S. Brown, R. G. W. Anderson, D. W. Russel, W. J. Schneider, *Annu. Rev. Cell Biol.* **1**, 1 (1985); A. Bansal and L. M. Gierasch, *Cell* **67**, 1195 (1991); I. V. Sandoval and O. Bakke, *Trends Cell Biol.* **4**, 292 (1994).
15. S. Amigorena, J. Salamero, J. Davoust, W. H. Fridman, C. Bonnerot, *Nature* **358**, 337 (1992).
16. R. N. Mitchell *et al.*, *J. Exp. Med.* **181**, 1705 (1995); T. L. Stevens, J. H. Blum, S. P. Foy, L. Matsuuchi, A. L. DeFranco, *J. Immunol.* **152**, 4397 (1994).
17. C. Watts, P. A. Reid, M. A. West, H. W. Davidson, *Semin. Immunol.* **2**, 247 (1990).
18. T. Kaisho, F. Schwenk, W. Müller, K. Rajewsky, *Science* **276**, 412 (1997); G. Achatz and M. C. Lamers, *ibid.*, p. 409.

19. We thank L. Nitschke, M. C. Lamers and K. Rajewsky for communication of unpublished data; T. Brocker, K. Karjalainen, and T. Simon for single-chain vectors; and L. Leclercq and P. Nielsen for critical reading of this manuscript. Supported partly by grants from the Deutsche Forschungsgemeinschaft (Re 571-3-1 and SFB 364).

22 November 1996; accepted 25 February 1997

Effect of Transmembrane and Cytoplasmic Domains of IgE on the IgE Response

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B cells use immunoglobulin M (IgM) and IgD as antigen receptors, but after contact with antigen they can switch and use IgG, IgA, or IgE. In mice lacking the transmembrane and cytoplasmic domains of IgE, serum IgE is reduced by more than 95 percent and, after immunization, specific responses are negligible. In mice lacking most of the cytoplasmic tail of IgE, serum IgE levels are reduced by 50 percent and specific responses are reduced by 40 to 80 percent, without a clear secondary response. Thus, membrane expression is indispensable for IgE secretion in vivo, and the cytoplasmic tail influences the degree and quality of the response.

Immunoglobulin E contributes least to the serum immunoglobulins (Igs). Its specific function is not understood, although it is well known as the cause of allergic reactions (*1*). IgE, like other Igs, is also expressed as an integral membrane protein (mIgE) on B cells. The transmembrane segments of mIgs are 25 amino acids long, whereas the cytoplasmic domains vary in size from three residues [Lys-Val-Lys (KVK)] for mIgM and mIgD to 14 to 28 residues for other isotypes (*2*). The nature and effects of the signals generated by mIgs other than IgM and IgD are mostly unknown, but they may control affinity maturation, memory induction, and differentiation into plasma cells (*3*). To study the role of the transmembrane domain and cytoplasmic tail of mIgE, we made mouse lines that carried mutations in these domains in the germline ε gene, using the gene-targeting technique in embryonic stem (ES) cells (*4*, *5*) (Fig. 1). The ΔM1M2 line lacks the transmembrane and cytoplasmic domains of IgE, whereas the KVKΔtail line can only express a cytoplasmic tail of three amino acid residues (KVK), which is identical to the cytoplasmic domain of mIgM and mIgD.

Serum Igs in 7-week-old unprimed mice were measured (Fig. 2A). There was

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no difference in IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA titers between age- and sex-matched wild-type and mutant mice; however, serum IgE was reduced by 94 to 98% in ΔM1M2 mice and by 50% in KVKΔtail mice. Similar reductions were found in 3- and 6-month-old animals (Fig. 2B). Thus, the mutations have no effect on serum concentrations of Igs other than IgE.

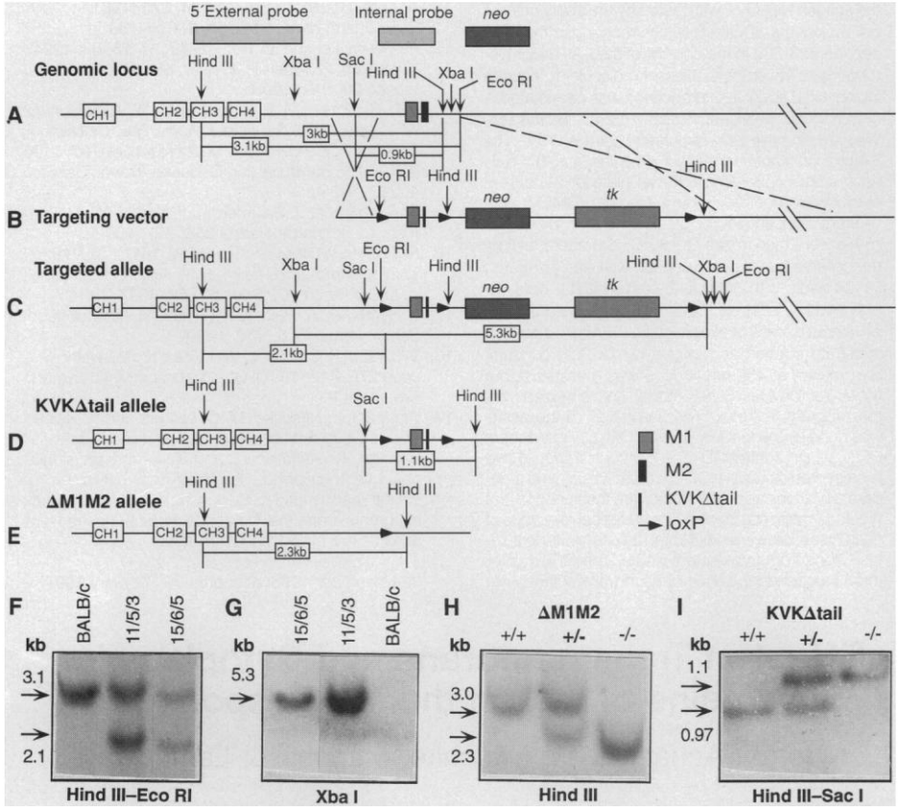

Fig. 1. Construction of the mutant mouse lines ΔM1M2 and KVKΔtail. (**A**) Organization of the *Cε* gene. The four constant-region exons are marked as CH1 to CH4. Membrane exons M1 and M2 are marked as indicated in inset at right. Selected restriction enzyme sites and the probes used for Southern blot analysis are shown. (**B**) Linearized targeting vector. (**C**) The *Cε* allele after primary targeting. (**D**) Cre-mediated recombination between the two loxP sites flanking the *neo* and *tk* genes results in the generation of the KVKΔtail allele. (**E**) Cre-mediated recombination between the most 5′ and 3′ loxP sites creates the ΔM1M2 allele. (**F** and **G**) Southern blot analysis of the primary targeting event. (F) Hind III–Eco RI–digested DNA was hybridized with the external probe. The sizes of wild-type and target fragments are indicated. The smaller (target) band is indicative of the presence of the singular loxP site (marked by the Eco RI site). Both clones 11/5/3 and 15/6/5 show equal intensities of the wild-type and target bands. (G) Hybridization of the Xba I–digested DNA with the *neo* probe shows singular integration events of clones 11/5/3 and 15/6/5. (**H** and **I**) Southern blot analysis of heterozygous and homozygous mice. (H) ΔM1M2 was confirmed by Hind III digestion and hybridization with the external probe. The sizes of the wild-type and targeted fragments are indicated. (I) KVKΔtail was confirmed by a Hind III–Sac I double digest and hybridization with a probe spanning the membrane exons. The mutant allele increases by the size of one singular loxP site (100 bp).

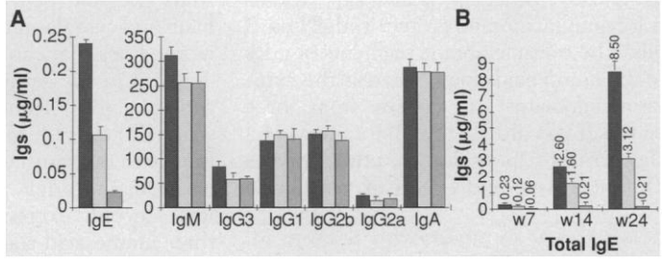

Fig. 2. Serum levels of immunoglobulins in wild-type (black bars), KVKΔtail (white bars), and ΔM1M2 (gray bars) mice. (**A**) Seven-week-old mice of each group were bled, and the sera of individual mice of each group were pooled (*6*) (**B**).The mice used for immunization experiments with DNP-OVA (see Fig. 3A) were bled before and after immunization. w7, 7-week-old mice, preimmune sera; w14 and w24, 14- and 24-week-old mice, bled 1 week and 11 weeks after the second booster immunization. Serum levels of total IgE were determined and are shown above bars. Error bars indicate standard errors.

The immune response of the mutant mice was assessed with two different immunization protocols. First, antibody titers were measured after mice were immunized with the T cell–dependent antigen 2,4-dinitrophenyl-ovalbumin (DNP-OVA) (*6*). Serum levels of specific IgG1 were comparable in wild-type and mutant mice (Fig. 3A). In ΔM1M2 mice, DNP-specific IgE antibodies were barely detectable, and in KVKΔtail mice titers were 50 to 80% lower than in control mice (Fig. 3B). Titers increased after the first booster, but a clear secondary response, as characterized by a strong and fast rise in specific antibody titer, was absent (Fig. 3B). Therefore, the transmembrane domain of IgE is indispensable for T cell–dependent IgE secretion, and the cytoplasmic tail influences the degree and quality of the response.

To determine whether the reduction in IgE titers in the mutant lines was caused by reduced levels of IgE production per cell or by a smaller number of cells that produce IgE, we measured the increase in the number of IgE-secreting cells 1 week after a third booster immunization with DNP-OVA (*7*). There was an average increase of 786 IgE-secreting cells in the wild-type mice, an average of 334 in KVKΔtail mice, and an average of 0 in ΔM1M2 mice. Therefore, the reduced IgE levels in the mutant mice reflected smaller numbers of IgE-secreting cells.

The second immunization protocol involved infestation with the helminth *Nippostrongylus brasiliensis* (*8*). *N. brasiliensis* induces robust IgG1 and IgE production, both through a dominant activation of type 2 T helper (T_H2) cells and a strong T cell–independent activation of B cells (*9*). Switch recombination to IgE and IgG1 is dependent on interleukin-4 (IL-4), whereas switch recombination to IgG2a is not induced by IL-4 (*10*, *11*). Serum IgG1 and IgG2a levels showed the expected pattern both in wild-type and mutant mice: an 8- to 10-fold increase in IgG1 levels at day 14 after infestation, and no increase in IgG2a titer. In wild-type mice (Fig. 3C), serum IgE rose from 300 ng/ml to 16 μg/ml by day 14 after infestation; in ΔM1M2 mice it rose from 20 ng/ml to 1.4 μg/ml; and in KVKΔtail mice it rose from 130 ng/ml to 8 μg/ml. After secondary infestation 11 weeks after the first challenge with *N. brasiliensis*, a strong and fast IgE response was seen in the wild-type mice (Fig. 3C), which is indicative of a memory response. The IgE response in the KVKΔtail mice was also substantial but was at 55% of the wild-type response. In the ΔM1M2 mice, IgE was now clearly

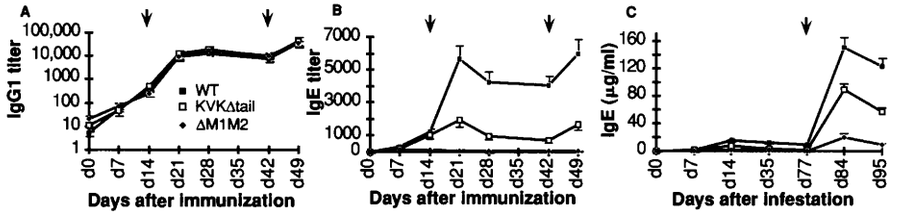

Fig. 3. Serum IgE responses in immunized mice. (**A** and **B**) Mice (six animals per group) were immunized with DNP-OVA and received booster immunizations after 2 and 6 weeks. Mice were bled at the indicated times and the sera of each group were pooled. Results are expressed as the serum dilution where half-maximum absorbance was obtained for IgG1 (A) and IgE (B). (**C**) Mice (five animals per group) were infested with *N. brasiliensis* at d0 and d77 and bled at the indicated times. Results are given as arithmetic means of serum levels of IgE. Bars indicate standard errors.

measurable; however, the response was sluggish and was reduced (13% of the wild-type response). The results indicate that the IgE response to *N. brasiliensis* also needs a specific interaction with the IgE antigen receptor complex on the B cell, accompanied by strong T_H2 cell activity.

To determine whether class switch to IgE was impaired by the targeting event, we stimulated isolated spleen cells of wild-type, ΔM1M2, and KVKΔtail mice in vitro with lipopolysaccharide (LPS) and IL-4 (*10*, *12*). As shown in Fig. 4, the concentrations of IgE and IgG1 in the culture supernatants were comparable in wild-type and mutant mice. These results imply that the reduced IgE titers found in both mutant lines are solely a reflection of the loss of biological activities associated with the transmembrane and cytoplasmic domains of IgE.

There are two possible explanations for these findings. First, signals generated via mIg are needed at all times, not only for the maturation process but also for the expansion of antigen-specific cells. Second, antigen presentation to T_H cells is necessary during an antibody response, and only the antigen receptor is capable of effective antigen capture for presentation. The hypotheses are not necessarily mutually exclusive. All Ig classes can associate with the Ig-α–Ig-β heterodimer, the signal-transducing unit of the B cell receptor

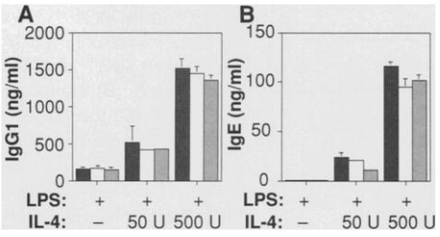

Fig. 4. (**A**) IgG1 and (**B**) IgE production of splenic B cells after in vitro stimulation with LPS and IL-4 at the indicated concentrations. Wild type, black bars; KVKΔtail, white bars; ΔM1M2, gray bars. Error bars indicate standard errors.

(*2*, *13*). Further, both the Ig-α–Ig-β sheath and the cytoplasmic tail of mIg (*14*, *15*) have been implicated in guiding receptor-bound antigen via the receptor to the antigen-processing compartments. Key residues for internalization are present in the tails in the form of a Tyr-X-X-Ile/Met motif, where X is any amino acid (*15*, *16*). These facts predict that the results we obtained in the KVKΔtail and ΔM1M2 lines can be extended to the IgG isotypes and perhaps to IgA. Indeed, Kaisho *et al.* (*17*) reach very similar conclusions in studying mice carrying matching mutations in the γ1 gene.

REFERENCES AND NOTES

1. B. J. Sutton and H. J. Gould, *Nature* **366**, 421 (1993).
2. M. Reth, *Annu. Rev. Immunol.* **10**, 97 (1992).
3. J. Jacob, G. Kelsoe, K. Rajewsky, U. Weiss, *Nature* **354**, 389 (1991); R. Küppers, M. Zhao, M. L. Hansmann, K. Rajewsky, *EMBO J.* **12**, 4955 (1993); C. Berek, A. Berger, M. Apel, *Cell* **67**, 1121 (1991); G. Kelsoe, *Immunol. Today* **16**, 324 (1995); R. Ahmed and D. Gray, *Science* **272**, 54 (1996).
4. K. R. Thomas and M. R. Capecchi, *Cell* **51**, 503 (1987); S. L. Mansour, K. R. Thomas, M. R. Capecchi, *Nature* **336**, 348 (1988).
5. The target vector consists of 7 kb of genomic DNA, spanning a region from 800 base pairs (bp) upstream to 6 kb downstream of the membrane exons M1 and M2. Polymerase chain reaction (PCR)–based site-directed mutagenesis was used to introduce a base exchange in the third codon of exon M2 (TGG to TGA, resulting in Trp to stop), followed by a frame shift. Correct mutagenesis was confirmed by sequencing. A Sma I site 3′ of exon M2 was used to introduce a selection cassette consisting of the neomycin resistance gene and the herpes simplex virus thymidine kinase gene (*4*), flanked by Cre recombinase recognition (loxP) sequences [B. Sauer, *Methods Enzymol.* **225**, 890 (1993); H. Gu, Y. R. Zou, K. Rajewsky, *Cell* **73**, 1155 (1993)] (a gift of J. Pelkonen). The third singular loxP segment was introduced 400 bp upstream of exon M1 in a Bal I site. The loxP segment was isolated from vector pBS112SX (a gift of B. Sauer and modified by J. Pelkonen). Correct orientation of the loxP segments was confirmed by sequencing. BALB/c ES cells (1×10^7) [N. Noben-Trauth, G. Köhler, K. Bürki, B. Ledermann, *Transgenic Res.* **5**, 487 (1996)] were transfected with 30 μg of linearized target vector and were cultured and treated as described [L. Nitschke, M. Kopf, M. C. Lamers, *BioTechniques* **14**, 914 (1993); L. Nitschke, in *Immunology Methods Manual*, I. Lefkovitch, Ed. (Academic Press, London, 1997), p. 218]. Targeting events were confirmed by Southern (DNA) blotting, using 5′ external and *neo* probes (Fig. 1, F and G). For site-specific recombination, 5×10^6 targeted ES cells were transfected with 20 μg of supercoiled pMC-CreN plasmid (a gift of H. Gu) that was optimized for efficient nuclear localization. After 10 days, resistant clones were screened by PCR. Cre-mediated recombination led to a small (Fig. 1D) and a large (Fig. 1E) deletion. The latter event was preferred with a ratio of 9:1 to the small deletion encompassing only the selection cassette. Correct recombination was confirmed by Southern blot analysis (Fig. 1, H and I). To confirm correct mutation, the mutant and wild-type alleles were recloned by PCR and sequenced. Mutated clones of both types were injected into blastocysts, and germline transmission was obtained for both. The traits were bred to homozygosity, yielding the KVKΔtail and ΔM1M2 lines.
6. Mice were immunized subcutaneously (sc) with 20 μg of DNP12-OVA precipitated in alum with *Bordetella pertussis*. Fourteen and 42 days later, the mice received a booster immunization with 20 μg of DNP12-OVA sc and 20 μg of DNP12-OVA intraperitoneally (ip). Serum was taken at day 0 (d0), d7, d14, d21, d35, d42, and d49. Sera were pooled, and DNP-specific IgG1 and IgE antibodies were detected with an enzyme-linked immunosorbent assay (ELISA). IgE titers were established after depletion of the sera with ProteinG [M. C. Lamers and P. Yu, *Immunol. Rev.* **148**, 71 (1995); P. Yu, M. Kosco-Vilbois, M. Richards, G. Köhler, M. C. Lamers, *Nature* **369**, 753 (1994)]. For some, but not all data points, measurements of individual mice were made to validate the use of pooled sera. The measurements in individual sera were in full agreement with those obtained in pooled sera.
7. For a modified spot ELISA [J. D. Sedgwick and P. G. Holt, *J. Exp. Med.* **157**, 2178 (1983)], microtiter plates were coated with rat monoclonal antibody (mAb) 84-1C to IgE and blocked with 1% bovine serum albumin. Serial dilutions of spleen cells were added to the wells in complete Iscove's medium and were cultured for 18 hours. Plates were washed and incubated with alkaline phosphatase–conjugated rat mAb EM95.3 to IgE. Spots were developed with the substrate BCIP (5-bromo-4-chloro-3-indolyl-phosphate) in 0.6% low-melting agarose.
8. Eight-week-old mice were infested sc with 750 stage-3 larvae of *N. brasiliensis* (a gift of M. Kopf). Serum was taken before infestation (at d0) and at d7, d14, and d35. Mice received a second dose of 750 larvae at d77 and were bled at d77, d84, and d95. Total serum IgE, IgG1, and IgG2a were determined by ELISA.
9. P. Lebrun and H. L. Spiegelberg, *J. Immunol.* **139**, 1459 (1987); N. E. Street *et al.*, *ibid.* **144**, 1629 (1990).
10. R. L. Coffman *et al.*, *ibid.* **136**, 4538 (1986).
11. M. Kopf *et al.*, *Nature* **362**, 245 (1993).
12. Spleen cells of two 8-week-old mice were pooled. After isotonic lysis of erythrocytes with Gey's solution, splenic cells were incubated at a density of 10^6 cells/ml with LPS (15 μg/ml, strain EH100; a gift of C. Galanos) and IL-4 (50 and 500 U/ml; Pharmingen, San Diego, CA) for 6 days, and supernatants were analyzed by ELISA for the presence of IgE and IgG1.
13. A. R. Venkitaraman, G. T. Williams, P. Dariavach, M. S. Neuberger, *Nature* **352**, 777 (1991).
14. K. J. Patel and M. S. Neuberger, *Cell* **74**, 939 (1993).
15. P. Weiser, R. Müller, U. Braun, M. Reth, *Science* **276**, 407 (1997).
16. I. V. Sandoval and O. Bakke, *Trends Cell Biol.* **4**, 292 (1994).
17. T. Kaisho, F. Schwenk, W. Müller, K. Rajewsky, *Science* **276**, 412 (1997).
18. Animal experiments were conducted in accordance with guidelines provided by the German law on experimentation with live animals. We thank K. Rajewsky and M. Reth for the exchange of data before publication, C. Westphal and S. Meier for blastocyst injection, H. Mittelstädt for technical assistance, P. Yu for the ε germline locus DNA, and R. Carsetti for reading the manuscript and stimulating discussions. G.A. was supported by a stipend of the Erwin-Schrödinger-Foundation.

22 November 1996; accepted 10 February 1997