

Interleukin 4 Gene-defective Mice Reconstituted with Wild-type Bone Marrow Fail to Produce Normal Immunoglobulin E Levels

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Summary

The ability to reconstitute interleukin (IL)-4^{-/-} mice with bone marrow of IL-4^{+/+} mice was investigated. The absence of the IL-4^{-/-} gene in donor or recipient cells did not impair the reconstitution. All immunoglobulin (Ig) subsets occurred at normal serum levels except for IgE and to some extent IgG1. IgE production did not recover in the reconstituted mice over prolonged time. However, these mice were competent for IgE production, because a single intrasplenic injection of IL-4 restored IgE levels, which then remained constant. Wild-type mice reconstituted with wild-type bone marrow constantly had IgE serum levels comparable to untreated animals. In wild-type mice reconstituted with IL-4^{-/-} bone marrow, IgE levels dropped gradually and disappeared by week 12. We make three unrelated but nonetheless important conclusions: (a) (immunoregulation) the tightly regulated IL-4 gene should be expressed constantly in low amounts (and with apparent absence of antigen stimulation) to keep the normal threshold of IgE; (b) (ontogeny of the immune system) an early unidentified source of IL-4 must be postulated which is lost in adult mice; and (c) (bone marrow transfer/gene therapy) under certain circumstances, the genotype of the recipient influences the reconstitution.

The production of immunoglobulin (Ig)E is under the strict control of IL-4 (1–3). IL-4 is expressed by bone marrow-derived cells (1). Its expression is tightly regulated and considered to be essentially after antigen exposure. The main source of IL-4 is CD4⁺ T cells of the Th2 subtype, which paradoxically require IL-4 to be induced (1). It has been suggested that NK1.1⁺ T cells initially provide IL-4 (4). However, the relative contribution of Th2 CD4⁺ T cells and NK1.1⁺ T cells for IL-4-regulated immune responses and the time point of production of IL-4 by either cell population *in vivo* are far from clear (4–10). The initial production and contribution to immune-regulated processes of IL-4 have been studied *in vitro* and *in vivo* after exposure of animals to antigens known to be strong inducers of IL-4. Yet IgE is constantly present in the serum in considerable amounts (a few hundred nanograms per milliliter) even in the absence of apparent antigen. Its half-life in serum is relatively short (5–12 h) (11–13). Therefore, new IgE should be produced constitutively. Since the half-life of IL-4 is even shorter (19 min after intravenous administration) (14), one should postulate that IL-4 is also constitutively produced. We prove here that this is indeed the case, because in normal mice reconstituted with bone marrow from IL-4^{-/-} mice, serum IgE levels declined during donor cell reconstitution.

Vice versa, if IL-4^{-/-} mice are reconstituted with IL-4^{+/+} bone marrow, the expectation would be that the mice do establish normal IgE levels. This result would meet the finding of others that reconstitution of gene-deficient mice (e.g., genes for apolipoprotein E, β -glucuronidase, complement receptor CR2, TNF/lymphotoxin, or GM-CSF/IL-3/IL-5 common receptor) with wild-type bone marrow restores the normal phenotype (15–20). Surprisingly, IL-4^{-/-} mice reconstituted with wild-type bone marrow remained unable to produce the threshold IgE level, and thus are defective for IL-4 production, even though they possess the ability to secrete IL-4 and IgE. Our explanation is that the cells initially producing IL-4 cannot be transferred with adult bone marrow, dating initial IL-4 production back in ontogeny.

Materials and Methods

Mice and Bone Marrow Transfer. C57BL/6 (IL-4^{+/+}) mice were obtained from Bomholtgaard Breeding & Research Centre, Ry, Denmark. IL-4-deficient mice (IL-4^{-/-}) (2) were backcrossed for eight generations to C57BL/6 mice and then intercrossed. All mice were kept under specific pathogen-free conditions and shown to be free of all viruses, parasites, and bacteria (except for occasional *Pasteurella pneumotropica*) by repeated controlling. Bone marrow from 6–8-wk-old IL-4^{+/+} and IL-4^{-/-} female mice was

harvested from both femurs and tibiae, and $1-2 \times 10^7$ cells were injected intravenously into lethally irradiated (9.5 Gy) 6–8-wk-old female recipient mice. The following groups were included in the experiments: IL-4^{+/+→+/+} (wild-type bone marrow transplanted into wild-type mice), IL-4^{+/+→-/-}, IL-4^{-/-→+/+}, IL-4^{-/-→-/-}, and age-matched control animals. In two separate experiments, 18 animals per group were investigated.

ELISA for Serum Ig Detection. At different time points (2, 4, 8, 12, and 16 wk, and in a second experiment, 48 wk after reconstitution), IgE levels were determined comparing serially diluted serum with commercially available Ig standards. The following reagents were used: R35-72 as capture antibody; purified mouse IgE, clone 27-74, as standard; and biotinylated R35-118 as secondary antibody (all from PharMingen Europe, Hamburg, Germany). For detection, avidin-peroxidase followed by 2,2' azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; both from Sigma Chemical Co., Deisenhofen, Germany) was used according to the manufacturer's recommendations, and the color reaction was read at 405 nm with a microplate ELISA reader (MR 5000; Dynatech Deutschland GmbH, Denkendorf, Germany). After long-term reconstitution (5 mo), IgG2a, IgG2b, IgG3, IgM, and IgA were detected in the sera of recipient mice using the Ig isotyping kit according to the manufacturer's recommendations (PharMingen Europe), and IgG1 levels with G1-6.5 as capture antibody, purified mouse IgG1, clone 107.3 as standard, and biotinylated R8-140 as secondary antibody (all reagents from PharMingen Europe). Except for IgG1, the amount of serum Igs in experimental animals was expressed as a percentage of the serum Ig of age-matched control animals.

PCR Analysis for Presence of Host/Donor-type Blood Cells. 5 mo after reconstitution, 100 μ l peripheral blood from four mice of each group was collected by retroorbital puncture. DNA was prepared using Sorb® Twin Prep according to the manufacturer's recommendations (InViTek GmbH, Berlin, Germany). β -Actin primers were used to adjust to comparable concentrations of DNA for 30 PCR cycles amplifying a 330-bp fragment. To detect wild-type and knockout IL-4 alleles, the 5' primer (5'-gCT AgT TgT CAT CCT gCT CTTC) was located upstream, the 3' primer (5'-gCC gAT gAT CTC TCT CAA gTg) downstream of the inserted *neo^R* gene within the IL-4 gene locus. The primers detect a 1,200-bp fragment, provided the genomic DNA contains the *neo^R* gene, and a 95-bp fragment in animals without the *neo^R* gene.

Peripheral Blood FACS® Analysis of Reconstituted Mice. 5 mo after bone marrow transplantation, 5×10^5 peripheral blood cells of four mice from each group were stained with 0.5 μ g mAbs against B220, CD4, CD8, and GR-1 for 30 min on ice. Isotype-matched rat Ig was used as a control (all antibodies from PharMingen Europe). Stained cells were fixed on the Q-Prep workstation with ImmunoPrep reagents, and analyzed using a flow cytometer (EPICS-XL; Coulter Electronics GmbH, Krefeld, Germany).

Immunohistochemical Analysis of Cryosections and Bone Marrow Cytospins. Embedded organs (thymus, Peyers patches) of animals 8 mo after transplant were cut on a Microtom-Kryostat HM500 OM (Microm Laborgeräte GmbH Life Sciences International GmbH, Walldorf, Germany). Cytospins (Shandon, Frankfurt, Germany) of bone marrow cells were air-dried overnight and stored at -20°C until use. Cryosections and bone marrow cytopins were fixed in ice-cold acetone for 10 min, air-dried, and washed in PBS. All of the following steps were carried out in a humid chamber. For anti-CD1d staining, preparations were blocked with 5% normal goat serum in PBS for 30 min, stained with 2 μ g/ml anti-CD1d (PharMingen Europe) for 30 min, and washed twice in PBS. Bound anti-CD1d was detected by incubation

with Texas red-labeled goat anti-rat IgG (1:50; Serva Feih-biochemica, Heidelberg, Germany). As negative control, goat anti-rat IgG (TXRD) was used under the same conditions. For NK1.1 and V β 8.1, 8.2 TCR detection, preparations were blocked with 5% BSA in PBS and double-stained with 1 μ g/ml anti-NK1.1-PE plus 1 μ g/ml anti-V β 8.1, 8.2 TCR-FITC (PharMingen Europe) for 30 min. Isotype-matched Ig was included as negative control. Stained preparations were mounted with Kaiser's glycerol gelatin (Merck, Darmstadt, Germany) and analyzed on a fluorescence microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Infection with *Nippostrongylus brasiliensis*. Mice (three C57BL/6 and three reconstituted IL-4^{+/+→-/-} mice 6 mo after transplant) were injected subcutaneously with 500 third-stage *N. brasiliensis* larvae. The serum IgE levels were determined before and 12 d after infection by ELISA as described above.

Injection of IL-4. Three C57BL/6 and two reconstituted IL-4^{+/+→-/-} mice 6 mo after transplant were injected intrasplenically with 500 U IL-4, which is equivalent to ~ 50 ng (biological activity 10^7 U/mg; IC Chemikalien GmbH, Munich, Germany). The serum IgE levels were determined before, and 7, 15, 28, and 42 d after injection by ELISA as described.

Results

Bone Marrow Reconstitution of IL-4 Congenic Mice. Four groups of bone marrow-reconstituted animals were generated: IL-4^{+/+} mice reconstituted with IL-4^{+/+} bone marrow (IL-4^{+/+→+/+}); IL-4^{-/-} mice reconstituted with IL-4^{+/+} bone marrow (IL-4^{+/+→-/-}); IL-4^{-/-} mice reconstituted with IL-4^{-/-} bone marrow (IL-4^{-/-→-/-}); and IL-4^{+/+} mice reconstituted with IL-4^{-/-} bone marrow (IL-4^{-/-→+/+}). Age-matched untransplanted C57BL/6 animals served as controls. To test the reconstitution of mice by donor cells, recipients were analyzed by PCR for wild-type and disrupted IL-4 gene in peripheral blood, hematopoietic cell subset composition, and serum Ig levels 5 mo after bone marrow transfer.

PCR analysis was done with primers close to the inserted neomycin sequence in the IL-4 gene, thus amplifying a 1,200-bp fragment for the defective and a 95-bp fragment for the wild-type allele. 5 mo after reconstitution, nearly 100% of peripheral blood cells in all four experimental groups were of the donor type, comparing the IL-4^{+/+→-/-} and IL-4^{-/-→+/+} groups with the IL-4^{+/+→+/+} and IL-4^{-/-→-/-} groups (Fig. 1). Only in some mice of the IL-4^{+/+→-/-} group were a few recipient cells left. Furthermore, hematological recovery of recipients was shown by similar numbers of B220⁺, CD4⁺, CD8⁺, and GR-1⁺ cells in the peripheral blood among the different groups (data not shown). Additionally, mice were analyzed 7 mo after transplant for the presence of NK1.1⁺ and V β 8.1, 8.2 TCR⁺ cells in the bone marrow and CD1d⁺ cells in the Peyers patches and thymus. This was done because NK1.1⁺ T cells preferentially using these TCRs occur at a comparably high frequency in bone marrow, are restricted to the CD1d antigen, and could be important for IL-4 production in the transplanted mice (4). Regardless of the genotype of the donor or recipient, NK1.1⁺/V β 8.1, 8.2 TCR⁺ and CD1d⁺

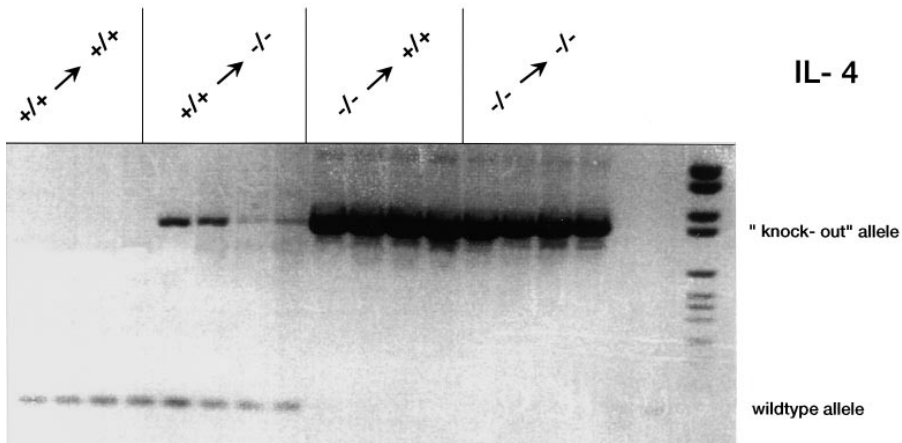


Figure 1. Peripheral blood cells of bone marrow-transplanted IL-4 congenic mice are of the donor cell type. 5 mo after reconstitution, four animals of each transplanted group were examined for host or donor cell types in peripheral blood. After equalization with β -actin primers (data not shown), genomic peripheral blood DNA was subjected to PCR using primers located in the vicinity of the inserted *neo^R* gene. DNA containing the *neo^R* gene gives rise to a 1,200-bp band and wild-type DNA to a 95-bp band.

cells could easily be detected in the investigated specimen (data not shown). Additional evidence for reconstitution with functional donor cells was found by determination of serum Ig isotypes IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM in long-term (5 mo) reconstituted mice (Fig. 2). All isotypes except for IgG1 were found in quite similar amounts in mice of all experimental groups. In mice of the IL-4^{-/-}→^{-/-} group, IgG1 was strongly reduced, but in IL-4^{+/+}→^{+/-} and IL-4^{-/-}→^{+/+} animals, a statistically insignificant IgG1 reduction was observed. Whether this is related to IL-4 has not been investigated. Taking the data together, there is no reason to assume that the absence of the IL-4 gene in either donor or recipient cells greatly impaired bone marrow reconstitution.

Serum IgE Levels Require the Continuous Presence of a Functional IL-4 Gene in Bone Marrow-derived Cells. We followed IgE serum levels in the transplanted mice over time, considering that IgE production is a direct reflection of IL-4 expression (1–3). Normal untransplanted C57BL/6 mice contained ~100 ng/ml IgE in serum at the age of 6–8 wk, which increased to an average of 400 ng/ml over a period of 3–4 mo (Fig. 3). IgE levels in mice of the IL-4^{+/+}→^{+/+} group at all time points closely resembled that in age-matched C57BL/6 control animals. Mice from the IL-4^{-/-}→^{-/-} group began and ended with no detectable IgE in the serum (detection limit 20 ng/ml). Mice in the IL-4^{-/-}→^{+/+} group had normal serum IgE levels until 4 wk after transplantation, which then dropped and disappeared completely in all animals by week 12 (Fig. 3). Because the only difference between the IL-4^{+/+}→^{+/+} and IL-4^{-/-}→^{+/+} groups is the presence or absence of a functional IL-4 gene in donor cells, we make three conclusions: (a) IL-4 is indeed only produced by bone marrow-derived cells and is mandatory for IgE production; (b) recipient cells in the IL-4^{-/-}→^{+/+} group seem to produce IL-4 continuously early after irradiation until they are replaced by donor cells at week 12 at the latest; and (c) the constant IgE levels in the IL-4^{+/+}→^{+/+} group can best be explained by a constitutive IL-4 production in the mice. All animals were kept under specific pathogen-free conditions and had no apparent contact with bacteria, viruses, or parasites, though this does not ex-

clude the possibility that the measured IgE was induced by exogenous antigens undetectable by us.

Bone Marrow-derived Cells from Adult IL-4^{+/+} Mice Are Unable to Induce Threshold IgE Levels in IL-4^{-/-} Recipients. Considering the results with mice from the IL-4^{-/-}→^{+/+}

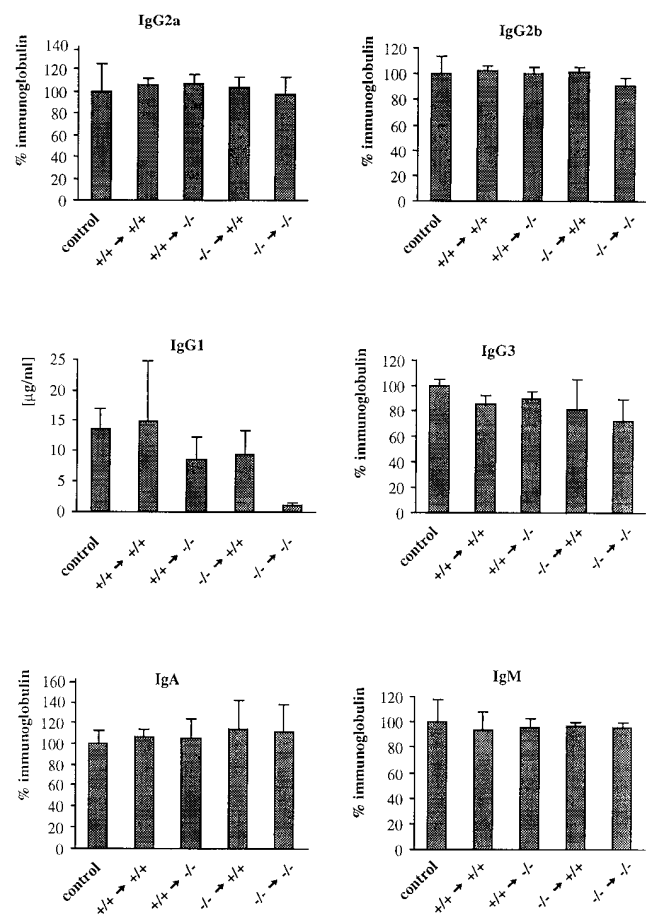


Figure 2. Analysis of serum Ig levels in bone marrow-reconstituted IL-4 congenic mice. 5 mo after reconstitution, serial dilutions of mouse sera were analyzed in an Ig-isotyping ELISA for IgM, IgG3, IgG1, IgG2b, IgG2a, and IgA. Except for IgG1, serum Ig amounts for age-matched control animals were set at 100%. Data represent mean \pm SD of eight animals.

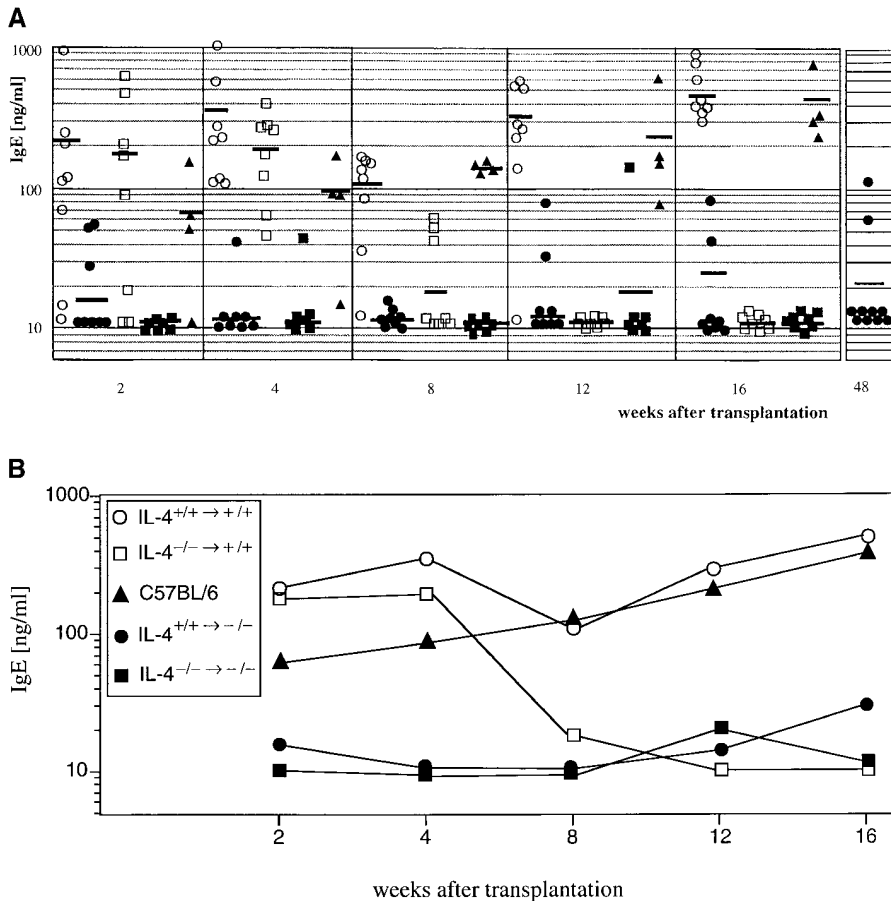


Figure 3. Serum IgE levels of bone marrow-reconstituted IL-4 congenic mice. Serum IgE levels were determined by an IgE-specific ELISA at indicated time points, comparing serial dilutions of sera with a commercial IgE standard. Groups of animals included were as follows: IL-4^{+/+}→+/+ (○), IL-4^{+/+}→-/- (●), IL-4^{-/-}→+/+ (□), IL-4^{-/-}→-/- (■), and age-matched control animals (▲). Shown are data from (A) eight individual animals, including the mean value for each group (black bar) up to 16 wk after transplantation. Similar results were obtained in a second experiment with 10 animals in each group. A third group of IL-4^{+/+}→-/- mice was established, and IgE serum levels were determined after 48 wk. (B) Mean IgE levels of mice shown in A. Detection limit of the IgE ELISA was 20 ng/ml.

group, one would expect that IL-4^{+/+}→-/- mice start to make IgE as a result of the functional IL-4 gene in bone marrow-derived cells by week 12 at the latest. However, IgE levels in mice of this group remained undetectable in almost all animals until week 16 and beyond after transplantation. Fig. 3 shows the serum IgE levels from one experiment using eight mice for each experimental group for up to 16 wk. In a second experiment, 10 animals of the IL-4^{+/+}→-/- group were measured for serum IgE levels 48 wk after transplantation. Again, IgE was undetectable in serum of most animals (8/10). However, as we have seen above, IL-4^{+/+} bone marrow-derived cells are very well able to produce IgE, if transplanted into IL-4^{+/+} hosts where in the early reconstitution phase, IL-4 is probably provided by the host. We conclude that IL-4^{+/+} bone marrow-derived cells do not have the ability to induce threshold IgE levels in IL-4^{-/-} hosts.

IL-4^{+/+} Bone Marrow-derived Cells in IL-4^{-/-} Recipients Can Be Induced to Produce IgE. Despite the fact that IL-4^{+/+}→-/- mice seemed to be reconstituted in a normal way (see above) and IL-4^{+/+} bone marrow-derived cells were able to initiate IgE expression in an appropriate environment, we could not exclude a hematologic impairment resulting from secondary effects, e.g., defective IgE expression in mice of the IL-4^{+/+}→-/- group resulting from par-

tial graft failure in the IL-4^{-/-} hosts undetectable by us. Therefore, mice from the IL-4^{+/+}→-/- group received a single intrasplenic injection of 500 U IL-4, and IgE serum levels were followed over time (Fig. 4). Within 7 d, IgE was detectable in serum, and at day 14 after IL-4 injection, IgE levels were comparable to normal C57BL/6 mice treated in the same way. Remarkably, IgE levels remained constant throughout the experiment (day 42), which can best be explained by endogenous IL-4 induced through exogenous IL-4, which subsequently regulated a normal threshold of IgE in serum. Additionally, 6 mo after reconstitution, IL-4^{+/+}→-/- animals were challenged with *N. brasiliensis* larvae, a strong natural IL-4 inducer. This led to an ~10-fold increase in serum IgE in normal mice after 12 d (Fig. 5). In IL-4^{+/+}→-/- mice, similar IgE levels were reached. Thus, defective IgE production in mice carrying a functional IL-4 gene in bone marrow-derived cells can be overcome by a strong IL-4-inducing stimulus.

Discussion

The question of which mechanism initiates IL-4 expression and subsequent IgE production has been studied mainly after antigenic stimulation. The stimuli used were strong inducers of IL-4 production, such as nematodes (1),

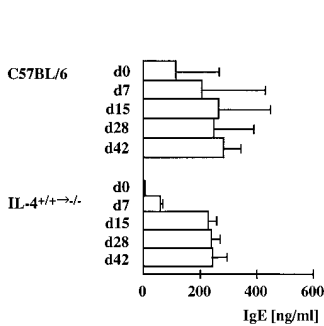


Figure 4. Defective IgE production in IL-4^{+/+}>-/- mice can be rescued by exogenous IL-4. Wild-type and IL-4^{+/+}>-/- bone marrow-reconstituted mice (6 mo after transplant) were injected intrasplenically with 500 U (~50 ng) IL-4, and serum IgE levels were determined before (d0), 7, 15, 28, and 42 d (d42) after injection. Data represent mean \pm SD of three and two mice, respectively.

polyclonal stimuli like anti-CD3 and anti-IgD (21), chronic antigen exposure (5), or adjuvant preferentially inducing Th2 responses such as alum (5, 22). Depending on the experimental system, CD4⁺ T cells (5), NK1.1⁺ T cells (21), or eosinophils (23) were responsible for initial IL-4 production. We addressed this question from a different point of view, considering that serum IgE levels are present in young animals without experimental antigenic stimulation. It has been shown that serum IgE is undetectable in nonimmunized IL-4^{-/-} mice and that even nematode infection fails to induce IgE expression in the absence of a functional IL-4 gene (2, 3). Therefore, we purposely measured serum IgE levels in the bone marrow-reconstituted animals, because it directly reflects IL-4 expression and because IL-4 is difficult to measure in naive animals. We used low IgE responder C57BL/6 mice, which in a pathogen-poor environment contain 70 ng/ml IgE in serum at the age of 6–8 wk, increasing to 400 ng/ml at the age of 5–6 mo on the average. Using bone marrow chimeras with IL-4^{-/-} mice, we showed that these IgE levels depend completely on the ability of the mice to express IL-4, and that their maintenance depends on the continuous presence of a functional IL-4 gene in bone marrow-derived cells. This was shown in mice of the IL-4^{-/-}>+/+ group, where IgE serum levels declined rapidly, proportional to replacement of IL-4-competent by IL-4-incompetent cells. Thus, taking into account sustained IgE serum levels in the IL-4^{+/+}>+/+ mice over a long time period and the comparably short half-lives of both IgE (11–13) and IL-4 (14), it becomes apparent that IL-4 should be constitutively expressed even though the animals were not exposed to any overt antigenic stimulus. It is not known which IL-4-producing cells are responsible for threshold IgE serum levels. IgE expression in mice of the IL-4^{-/-}>+/+ group must not necessarily reflect B cells induced by the recipient's IL-4 to switch to IgE expression after irradiation. Recently, it has been shown that the life-time of plasma cells which secrete ovalbumin-specific IgG1 antibodies in the bone marrow can be as long as 90 d without DNA synthesis (24). The observation that IgE serum levels in IL-4^{-/-}>+/+ mice disappeared within ~8 wk indicates that the half-life of IgE-secreting plasma cells in bone marrow-reconstituted mice is shorter.

Our most important finding was that IL-4^{-/-} mice re-

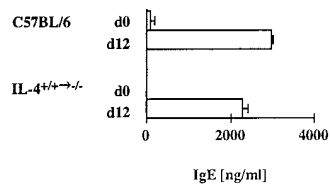


Figure 5. Defective IgE production in IL-4^{+/+}>-/- mice can be rescued by *N. brasiliensis*. Wild-type and IL-4^{+/+}>-/- reconstituted mice 7 mo after transplant were injected subcutaneously with third-stage larvae of *N. brasiliensis*, and serum IgE levels were analyzed before (d0) and 12 d (d12) after injection. Data represent mean \pm SD of three animals.

constituted with IL-4^{+/+} bone marrow failed to establish normal IgE serum levels. Two control experiments showed that this defect was caused by the absence in adult bone marrow of cells from which the initially IL-4-providing cells derive. In the first, if IL-4^{+/+} bone marrow was transplanted into IL-4^{+/+} mice, the animals continued to produce normal serum IgE levels. Therefore, IL-4^{+/+} bone marrow-derived cells are able to produce IL-4 and IgE. In the IL-4^{+/+} recipient mice, IL-4 and concomitantly IgE might be produced for a certain period after lethal irradiation and bone marrow transfer. This period can be estimated in the IL-4^{-/-}>+/+ group of mice, lasting between 6 and 8 wk after transplantation. During replacement of recipient by donor cells, donor bone marrow-derived cells are educated by the recipient's IL-4 to produce IL-4 themselves and subsequently to maintain threshold serum IgE levels. In the second control experiment, in mice of the IL-4^{+/+}>-/- group, defective IgE production could be rescued by a single injection of IL-4, ruling out partial graft failure as the reason for the defect. Because these mice continued to produce IgE throughout the experiment (42 d), the exogenous IL-4 should have induced IL-4 expression in donor-derived cells. Once initiated, IL-4 expression acts in an autostimulatory fashion. Therefore, our results demonstrate that bone marrow cells of adult animals are unable to give rise to cells from which IL-4 first originates, and thus are functionally not pluripotent. This raises the question of when and by which cells initial IL-4 is provided. We offer two not mutually exclusive explanations. First, IL-4 is expressed during early development. This is supported by the observation that IL-4 mRNA as well as IL-4 receptor mRNA could be detected in 10.5- and 12.5-d-old embryos (25). In extracts of some tissues (e.g., liver) of 12.5-d-old fetuses, IL-4 protein could also be detected. Furthermore, hematopoietic progenitor cells differentiated in vitro from embryonic stem cells express IL-4 and IL-4 receptor mRNA (26). Second, IL-4 is provided by the mother. This alternative is supported by the observation that pregnant mice (27–29) and women (30) produce increased amounts of IL-4, and IL-4 mRNA is detected in uterine decidual plus placental tissue at days 8.5–12.5 of gestation (25). We currently analyze these two possibilities. However, regardless of whether induction of the IL-4-regulated network results from “maternal imprinting” or from the expression of IL-4 by embryonic cells, the fetus is exposed to IL-4, and the initial source of IL-4 is not necessary any more in the adult because the system keeps itself alive.

IgE production in mice of the IL-4^{+/+→-/-} group can also be rescued by challenge with larvae of *N. brasiliensis*, further supporting that normal hematopoietic reconstitution had occurred in the animals. Even though it has been shown that the IL-4 requirement for IgE production can be bypassed by some antigens such as *Plasmodium chabaudi* or a mouse retrovirus (31, 32), IgE production in the bone marrow-reconstituted mice after nematode exposure probably occurs in an IL-4-dependent way, because IL-4^{-/-} mice fail to produce detectable amounts of IgE after nematode challenge (2, 3). However, we think it is unlikely that strong IL-4 inducers such as nematodes are the driving

force for establishing the IL-4-regulated immune system in early life.

For a variety of genetic diseases, bone marrow reconstitution with cells genetically modified to carry a functional copy of the defective gene is considered to be a therapeutic modality. Wild-type bone marrow transplantation into gene-deficient mice has been shown in several models to restore a normal phenotype (15–20). Our results differ in this regard and raise the caution that for certain genes, e.g., those involved in regulation of hematopoiesis, gene therapy approaches encounter problems not anticipated previously.

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