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Transplantation Tolerance to a Single Noninherited MHC Class I Maternal Alloantigen Studied in a TCR-Transgenic Mouse Model

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The mechanisms underlying tolerance to noninherited maternal Ags (NIMA) are not fully understood. In this study, we designed a double-transgenic model in which all the offspring’s CD8+ T cells corresponded to a single clone recognizing the Kb MHC class I protein. In contrast, the mother and the father of the offspring differed by the expression of a single Ag, Kb, that served as NIMA. We investigated the influence of NIMA exposure on the offspring thymic T cell selection during ontogeny and on its peripheral T cell response during adulthood. We observed that anti-Kb thymocytes were exposed to NIMA and became activated during fetal life but were not deleted. Strikingly, adult mice exposed to NIMA accepted permanently Kb+ heart allografts despite the presence of normal levels of anti-Kb TCR transgenic T cells. Transplant tolerance was associated with a lack of a proinflammatory alloreactive T cell response and an activation/expansion of T cells producing IL-4 and IL-10. In addition, we observed that tolerance to NIMA Kb was abrogated via depletion of CD4+ but not CD8+ T cells and could be transferred to naive nonexposed mice via adoptive transfer of CD4+CD25high T cell expressing Foxp3 isolated from NIMA mice. The Journal of Immunology, 2011, 186: 1442–1449.

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Abbreviations used in this article: GVH, graft-versus-host; IMA, inherited maternal Ag; MST, mean survival time; NE, never exposed to Kb; NIMA, noninherited maternal Ag; pc, postcoitum; pp, postpartum; Tg, transgenic; Treg, regulatory T cell.

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Similarly, Zhang and Miller (9) reported some tolerogenic effects of NIMA on semiallogeneic maternal skin transplants in mice. In this model, both pregnancy and breast-feeding were required to achieve long-term graft survival. In collaboration with Burlington’s group (10), we previously investigated the effects of NIMA on polyclonal T and B cell alloresponses and allotropic transplant rejection in mice. We reported that the majority of H-2<sup>b</sup>/H-2<sup>b</sup> offspring of semiallogeneic (H-2<sup>b</sup>/H-2<sup>b</sup>) mothers accept fully allogeneic DBA/2 heart grafts (graft survival > 180 d) (10). Strikingly, no signs of intimal thickening and fibrosis, which are characteristic features of chronic rejection, were detected in heart transplants collected from NIMA-exposed mice. In this model, long-term survival of heart transplants expressing NIMA was observed exclusively in offspring that had been both carried and breast-fed by a mother expressing NIMA (10). We also demonstrated a specific influence of NIMA on the development of offspring’s B lymphocytes in a BCR transgenic (Tg) model, distinct from the fate of self-reactive B cells in the same model (11, 12). Collectively, these studies underscore the potent tolerogenic effects of NIMA in allotransplantation. In contrast, Molitor-Dart et al. (13) have recently reported that, under certain circumstances, the presentation of NIMA can result in offspring’s sensitization rather than tolerization. However, the mechanisms by which NIMA actually drive the immune system toward transplant tolerance or rejection remain unclear. Elucidation of this question is likely to pave the way for the design of novel tolerance protocols in clinical transplantation.

In this study, we used a model in which a single NIMA is the MHC class I H-2<sup>K</sup> molecule in a K<sup>b</sup>-Tg mouse and the offspring express an anti-K<sup>b</sup> TCR transgene on CD8<sup>+</sup> T cells. We observed that the fetus’s anti-K<sup>b</sup> TCR Tg thymic T cells were exposed and activated to NIMA during pregnancy and neonatal life up to 3 wk of age, leading to the deletion of half of T cells during this period. The adult offspring displayed long-term survival of NIMA-expressing heart allotransplants. Tolerance to NIMA was mediated via the suppression of the proinflammatory response by anti-K<sup>b</sup> CD8<sup>+</sup> T cells and the activation/expansion of CD4<sup>+</sup>CD25<sup>high</sup> Foxp3<sup>+</sup> regulatory T cells (Tregs) recognizing the K<sup>b</sup> alloantigen. The implications of these findings for the design of tolerance protocols in allotropic transplantation are discussed.

**Materials and Methods**

**Mice and transplantsations**

Mice were bred and maintained at Massachusetts General Hospital and Institut Jacques Monod’s animal facilities under specific pathogen-free conditions. All animal care and handling were performed according to institutional guidelines. The day of the vaginal plug was considered as day 0.5 of gestation. CBK Tg (CBA/ca mice [H-2<sup>k</sup>] expressing a K<sup>b</sup> MHC class I transgene) were used as donors in heart transplants (14). Offspring of BM3.3 anti-K<sup>b</sup> TCR Tg male mice (15) and F1 (CBA/ca × CBK) females were used as NIMA (offspring that do not inherit K<sup>b</sup>) and IMA (offspring that inherit K<sup>b</sup> maternal Ag) recipient mice (Fig. 1). To separate NIMA and IMA offspring, we stained blood from orbital sinus with an anti-K<sup>b</sup> FITC mAb. No cells from NIMA were K<sup>b</sup><sup>+</sup>, whereas all the cells were found to express K<sup>b</sup> in IMA mice. Offspring of BM3.3 anti-K<sup>b</sup> TCR Tg male and CBK female mice were used as positive control animals for tolerance to K<sup>b</sup> (K<sup>b</sup> inherited as self: IMA). Offspring of BM3.3 anti-K<sup>b</sup> TCR Tg male and wild type CBA female mice (never exposed to K<sup>b</sup>; NE) were used as negative control animals (i.e., lack of tolerance to K<sup>b</sup>). NIMA, IMA, and NE mice were transplanted in the peritoneal cavity with a vascularized CBK (K<sup>b</sup>-Tg) heart using the microsurgical technique previously described by Corry et al. (16). Graft rejection was monitored by daily palpation of heart and confirmed by histological techniques. In some experiments, CD4<sup>+</sup> or CD8<sup>+</sup> T cells were depleted from recipient mice with anti-CD4 (GK1.5) and anti-CD8 (53.6.72) mAbs (1 mg given i.p. at days −3 and −1 pretransplant), respectively.

**Cell suspensions**

Cells were isolated from thymus and spleen of individual fetuses at 18.5 d postcoitum (pc) and neonates at 3.5 d postpartum (pp). Organs were gently pressed through a sieve using a syringe plunger and suspended in PBS containing 4% FCS and 0.1% sodium azide (PBS/FCS/NaN<sub>3</sub>). Viable cells were counted by trypan blue exclusion.

**Immunofluorescence staining and flow cytometric analyses**

Aliquots of 5 × 10<sup>5</sup> nucleated cells were incubated for 40 min at 4°C with an optimal amount of the following mAbs: anti-CD8 coupled to FITC, anti-CD4, anti-CD25, anti-CD44, anti-CD69, anti-CD62L, anti-CD25, anti-CD44, anti-CD69, and anti-CD62L. Viable cells were gated and examined on a FACS Calibur. The expression of Foxp3<sup>+</sup> regulatory T cells (Tregs) recognizing the K<sup>b</sup> alloantigen was assessed in positive control mice (IMA and CBK, negative control mice; NE, and experimental NIMA mice. Representative FACs profiles obtained with spleen cells are shown.

**Figure 1.** The model. A. To obtain NIMA and IMA mice, we mated Bm3.3 anti-K<sup>b</sup> TCR Tg male mice with (CBK [CBA, K<sup>b</sup>-Tg] × CBA) F1 female mice. The offspring, which inherited both the anti-K<sup>b</sup> TCR transgene and the K<sup>b</sup> transgene, were referred to as IMA mice. The offspring, which inherited the anti-K<sup>b</sup> TCR transgene but not the K<sup>b</sup> transgene, were referred to as NIMA mice. NE control offspring were obtained by mating BM3.3 anti-K<sup>b</sup> TCR Tg female mice with CBA male mice (these mice could never be exposed to K<sup>b</sup>). B. The expression of MHC class I glycoprotein K<sup>b</sup> was assessed in positive control mice IMA and CBK, negative control mice NE, and experimental NIMA mice. Representative FACs profiles obtained with spleen cells are shown.
anti-CD4 coupled to PE, anti-CD25 coupled to FITC, anti-CD44 coupled to PE, anti-TCR β-chain coupled to PE (all purchased from Pharmingen), and anti-BM3.3 clonotype Ti98 prepared according to conventional techniques and coupled to biotin. Cells were washed twice in PBS/FCS/NaN₃. Biotinylated Ab was revealed by incubating cells for 20 min at 4°C with streptavidin-PE or streptavidin-allophycocyanin. After washing, cells were analyzed on a CyAn LX flow cytometer (DakoCytomation) equipped with 488- and 635-nm lasers. The cell populations analyzed were gated on the viable lymphoid cell population on the basis of forward and side scatter criteria. When possible, at least 10⁶ Ti98+ cells were analyzed from each sample.

**T cell assays**

The deletion of anti-Kᵇ TCR Tg T cells was monitored with an anti-clonotypic mAb (Ti98) using FACS analysis. The frequencies of type 1 and 2 cytokine-producing T cells responding to Kᵇ via the direct allore cognition pathway were determined using an ELISPOT method as previously described (4).

**Morphology analyses**

Cardiac transplants were fixed in 10% buffered formalin, embedded in paraffin, coronally sectioned, and stained with H&E for evaluation of cellular infiltrates and myocyte damage (acute rejection) by light microscopy. For assessment of chronic rejection, cardiac grafts were stained with Verhoeff’s elastin (vessel arteriosclerosis scoring) or Mason’s trichrome (evaluation of fibrosis). Arteriosclerosis was assessed by light microscopy, and the percentage of luminal occlusion and intimal thickening was determined using a scoring system, as previously described (17). Only vessels that display a clear internal elastic lamina were included in morphometric analysis (five to seven vessels per section). All arteries were scored by at least two examiners in a blinded fashion.

**Statistical analyses**

Statistical analyses were performed using STATView software (Abacus Concepts, Berkeley, CA). The p values were calculated using paired t test. A p value <0.05 was considered statistically significant.

**Results**

In this study, we used a mouse Tg model in which the NIMA is an MHC class I transgene, Kᵇ, and all the offspring’s T cells express an anti-Kᵇ TCR transgene (BM3.3 Tg mice; Fig. 1). The TCR from CD8+ T cells of BM3.3 mice recognize intact Kᵇ MHC class I molecules bound to an 8-mer peptide (INFDFNTI) called BM1 (direct allore cognition). Both CBK (Kᵇ Tg) and Bm3.3 (anti-Kᵇ TCR Tg) mice used as parents were engineered in CBA/Ca (H-2ᵏ) mice. To study the NIMA effect, we mated heterozygous female mice (CBA × CBK) F1 with homozygous Bm3.3 TCR Tg males. In this setting, all the offspring inherit the TCR Tg from their father and express anti-Kᵇ TCR on their CD8+ T cells. Half the offspring are expected to inherit the Kᵇ transgene from their mother and are referred to as IMA mice. The other half of the offspring should not inherit Kᵇ from their mother; these mice are called NIMA mice. In addition, non-Tg CBA females were mated with Bm3.3 anti-Kᵇ TCR Tg males. In the absence of a Kᵇ transgene in the mother, the resulting offspring do not inherit Kᵇ and are, therefore, never exposed to it and are referred to as NE mice. The design of NIMA, IMA, and NE mice is depicted in Fig. 1A. The phenotype of NE, NIMA, and IMA offspring was ascertained by staining splenocytes using anti-Kᵇ Abs. As expected, the splenocytes of CBK and IMA mice expressed MHC class I Kᵇ molecules, whereas the spleen cells from NE and NIMA mice did not display Kᵇ on their surface (Fig. 1B).

Next, we investigated the influence of NIMA on allotransplant rejection by the offspring. To test this, we transplanted NIMA mice with a CBK (Kᵇ Tg) allogeneic heart. Acute and chronic rejections of the cardiac allografts were monitored by palpation and histological techniques. In these experiments, NE mice, which are never exposed to Kᵇ, and IMA mice that inherit Kᵇ from their mothers were used as control recipients for rejection and tolerance, respectively. As expected, NE mice rejected CBK heart transplants in an acute fashion (12 ± 4 d; Fig. 2A), whereas IMA mice accepted their transplants indefinitely (Fig. 2A). Twenty-two of the 25 NIMA mice tested (>80%) accepted CBK heart transplants permanently. Three mice rejected their transplants, although in a markedly delayed fashion (40–60 d). As shown in Fig. 2B, massive infiltration and tissue damage were detected in the heart transplants of control NE mice tested 12 d after grafting. In contrast, in NIMA mice, histological examination of cardiac transplants performed 50 d after allograft placement revealed no inflammatory cell infiltrates and a well-preserved tissue architecture (Fig. 2B). Therefore, NIMA mice are tolerant to Kᵇ allogeneic CBK heart transplants. This suggests that although NIMA mice did not inherit Kᵇ MHC class I Ag from their mothers, they had been exposed to this allo-MHC Ag during their development.

It was possible that tolerance to Kᵇ allografts in NIMA mice was due to the deletion of anti-Kᵇ TCR Tg T cells during thymic selection. To test this, we assessed the presence of TCR Tg T cells in the peripheral blood of adult mice by FACS using an anti-clonotypic mAb, Ti98. Control non-TCR Tg CBA (NE) and CBK mice displayed a normal polyclonal population of CD8+ cells, whereas the spleen cells from NE and NIMA mice tested individually.
T cells, which did not express the T98 clonotype (Fig. 3A), whereas virtually all the CD8+ T cells found in Bm3.3 TCR Tg mice were T98+ (Fig. 3B). Strikingly, no T98+ were detected in Bm3.3 mice, which had inherited Kβ from their mothers (IMA) (Fig. 3C). Therefore, NE mice did not delete their anti-Kβ TCR Tg T cells, whereas IMA mice in which Kβ represents a self-antigen deleted their anti-Kβ TCR Tg T98+ T cells. Most important, normal levels of T98+ T cells similar to those observed in control BM3.3 NE mice were found in NIMA mice (Fig. 3D). Therefore, deletion of anti-Kβ TCR Tg CD8+ T cells is not responsible for Kβ-specific tolerance in adult NIMA mice.

Next, we examined whether T cells from NIMA mice are exposed to Kβ alloantigens during thymic development. The thymin of NE, IMA, and NIMA mice were collected during fetal life at days 16.5 and 18.5 postcoitum (pc) (fetuses), at birth time, and 3.5 d pp (neonates). As shown in Fig. 4A, a few T cells were detected at day 16.5 pc in all mouse groups, but numbers were already reproductively decreased in NIMA fetuses. At day 18.5 pc and birth time, control NE mice displayed high numbers of Kβ-specific thymocytes, whereas none was found in IMA mice. This is consistent with a model in which BM3.3 T cells expanded in NE mice (positive selection), whereas they were deleted in IMA mice (negative selection). At this time point, TCR Tg T cells were detected in NIMA mice, although at half the frequency found in control NE mice. In turn, at day 3.5 pp, the number of anti-Kβ T cells had doubled in NIMA mice but remained significantly lower than that observed in NE mice (Fig. 4A), and the same observation could be made at 3 wk pp (data not shown). Virtually no anti-Kβ TCR Tg T cells were found at each of these time points in IMA mice, a result consistent with their clonal deletion (Fig. 4A). Altogether, these results indicate that NIMA mice are exposed to and affected by NIMA Kβ allo-MHC class I Ag during fetal life. To confirm this, thymocytes from NE and NIMA mice were permeabilized and tested at day 18.5 pc and 3.5 pp for their proliferation rate using propidium iodide. The FACS profiles presented in Fig. 4B show the frequencies of T cells in G0/G1 phase (left peak) and in S-M/G2 phase (right peak). The results show a marked increase in the proliferation rates in NIMA mice as compared with NE mice in both fetuses (4 versus 10%; p < 0.05) and neonates (18 versus 40%; p < 0.05). This observation further supports the view that, in NIMA mice, T cells are exposed to NIMA and activated to proliferate during fetal thymic development. This phenomenon occurred up to weaning age at the end of the transfer of maternal cells through suckling.

In another set of experiments, we compared anti-Kβ T cell-mediated alloresponses in adult NE, IMA, and NIMA mice (Fig. 5). T cells from the spleen of naive mice and mice transplanted with a CBK heart were isolated and placed in culture with allogenic irradiated CBK stimulator cells (MLR), a test that is traditionally used to detect direct alloreactivity. The frequencies of anti-Kβ alloreactive T cells producing type 1 (IL-2 and IFN-γ) and type 2 (IL-4 and IL-10) cytokines were measured using an ELISPOT assay as previously described (18). In naive control NE mice, ∼200 activated T cells per million T cells were found to produce IL-2, IFN-γ, and IL-4, but no IL-10, which is consistent
with the frequencies previously reported for a primary MLR. As expected, these frequencies were much greater (>1000 spots/million) in NE mice that had been transplanted with a CBK heart, whereas no T cells producing IL-10 were detected in these mice. In contrast, no activated T cells producing IL-2, IFN-γ, and IL-4 were detected in both naive and transplanted IMA mice. Interestingly, however, a few T cells producing IL-10 were found in naive and transplanted IMA mice. In NIMA mice, although no alloreactive T cells producing IL-2 and IFN-γ were found, some T cells producing IL-4 were detected. In addition, IL-10–secreting anti-Kb T cells were detected in naive NIMA mice and particularly in NIMA mice that had received a cardiac allograft. These cytokines are traditionally secreted by type 2 (TH2/CT2) cells and Tregs. Our results also imply that, although most Ti98+ TCR Tg T cells had been deleted in developing IMA mice, some anti-Kb T cells producing IL-10 had escaped negative selection and could become activated in adults after exposure to Kb alloantigen.

Next, we investigated the mechanisms underlying transplantation tolerance in adult NIMA mice. First, NE, NIMA, and IMA mice were treated with depleting anti-CD4 or anti-CD8 Abs starting 3 d before transplantation with an allogeneic Kb+ CBK heart. This resulted in the near-complete depletion of CD4+ and CD8+ T cell subsets for more than 2 wk after Ab administration (data not shown). As shown in Fig. 6A, the depletion of CD8+ T cells resulted in long-term survival of Kb+ heart transplants in NE mice, whereas the anti-CD4 mAb treatment had no effect. This is consistent with the fact that CBK allografts placed in these BM3.3 Tg mice are rejected primarily by CD8+ TCR Tg anti-Kb T cells. Although some CD4+ T cells can be found in these mice, they apparently do not contribute to the rejection of Kb+ allografts. The majority (>80%) of nontreated NIMA mice either accepted Kb+ allotransplants or exhibited marked delayed rejection (>60 d post-transplantation; Fig. 6B). All NIMA mice treated with anti-CD8 Abs retained CBK cardiac transplants indefinitely, a result that is consistent with the observations made in NE mice. Most important, the majority of NIMA mice treated with anti-CD4 mAbs rejected CBK hearts between 10 and 30 d post-transplantation (Fig. 6B). Histological examination of the rejected transplants revealed massive inflammatory infiltrates and tissue damage typical of acute cellular rejection (data not shown). Therefore, deletion of CD4+ T cells in NIMA mice had abolished tolerance to Kb alloantigen. Surprisingly, we observed that depletion of CD4+ T cells in IMA mice induced the rejection of CBK heart transplants in ~50% of the mice. Therefore, tolerance to Kb can be broken in IMA mice, a result suggesting that clonal deletion of anti-Kb T cells is not the sole mechanism underlying tolerance induction and/or maintenance in these mice (data not shown).

The results obtained in NIMA mice with anti-CD4 mAbs prompted us to test whether these mice display CD4+ Tregs responsible for inducing or maintaining tolerance to Kb alloantigen. To address this question, we isolated CD4+CD25high and CD4+CD25− T cells from the spleens of NIMA mice by FACS sorting (>91% purity). More than 92% of CD4+CD25high T cells were Foxp3+, whereas the CD4+CD25− T cells did not express significant Foxp3 levels (data not shown). Each subpopulation was adoptively transferred (5 × 10⁶ cells given i.v.) into CBA naive mice 5 d before their transplantation with a CBK heart. As shown in Fig. 7, the mice administered with CD4+CD25− T cells from NIMA mice rejected CBK cardiac allografts in an acute fashion.

**FIGURE 5.** Frequencies of cytokine-producing T cells in naive and transplanted mice. The frequencies of alloreactive anti-Kb T cells secreting proinflammatory type 1 cytokines IL-2 (A), IFN-γ (B), and type 2 “regulatory” cytokines IL-4 (C) and IL-10 (D) were measured by ELISPOT. Spleen cells from nontransplanted (naive, white bars) and mice recipient of a CBK heart transplant (10 d post-transplant, solid bars) were collected and stimulated in vitro with irradiated CBK Kb+ Tg splenocytes (MLR). The results are presented as cytokine-producing spots per million T cells ± SD. The results are representative of four experiments each including two to three mice tested individually.
In contrast, adoptive transfer of five CBA mice with CD4+CD25high T cells collected from NIMA mice resulted in a significant increase (p = 0.02) of allograft survival in four of five mice (MST: 89 ± 18 d). Histo-
logical examination of the transplanted hearts revealed no signs of chronic allograft vasculopathy in these mice (data not shown). It is noteworthy that these adoptively transferred mice rejected acutely third-party BALB/c (H-2d) cardiac allografts (data not shown). In contrast, adoptive transfer of CD4+CD25high T cells, as well as CD4+CD25low T cells from NE mice, had no effect on graft rejection. Therefore, the tolerance to Kb can be adoptively transferred to CBA NE mice using CD4+CD25high T cells collected from the spleens of NIMA mice. In NIMA mice, anti-Kb TCR Tg T cells are not deleted and some Kb-specific CD4+ Tregs may be selected, which can ensure tolerance to Kb allografts. Interestingly, some modest but significant prolongation of allograft survival was also observed on transfer of CD4+CD25high from IMA mice (MST: 28 ± 5 d; p = 0.04). This result further supports the view that, in IMA mice, although CD8+ anti-Kb T cells are eliminated in the developing thymus, some CD4+ Tregs escape negative selection and can confer some protection against re-
jection of Kb+ allografts following adoptive transfer in naive CBA mice.

Discussion
Transplantation tolerance, defined broadly as long-term allograft survival in the absence of immunosuppressive treatment, is regularly achieved in nature during pregnancy. Mammalian pregnancy and subsequent nursing of the newborn appears to have a profound influence on the neonate's developing immune system that is retained in adulthood. In animal models, the passage of maternal cells and Ags during gestation and breast-feeding is thought to imprint long-term unresponsiveness of NIMA-specific inflam-
matory T cells in offspring. This phenomenon is clinically relevant as exemplified by the beneficial effects of matching donors and recipients for NIMA in human recipients of blood transfusion and kidney allotransplants (19). In addition, there is a body of evi-
dence suggesting that the presence of NIMA also influences the adult's susceptibility to autoimmune disorders (4, 20–22). Alto-
gether, these observations indicate that NIMA play a critical role in the establishment and regulation of the entire immune system. However, the mechanisms underlying the induction of a NIMA effect in the fetus and neonates and its maintenance in adults are not fully understood. Gaining insights into this question will set the path for the design of novel strategies for manipulating the immune system in health and disease.

The elucidation of the mechanisms underlying the NIMA effect has been difficult because of the fact that the precise nature of the NIMA and the T cell clones recognizing these maternal Ags are unknown. To overcome this, we designed a double-Tg model in which the mother’s NIMA and offspring’s anti-NIMA T cells were
well defined. In this model, all the offspring’s CD8+ T cells corresponded to a single clone recognizing the Kβ MHC class I protein. In contrast, the mother and the father of the offspring differed by the expression of a single Ag, Kβ, that served as NIMA. This allowed us to study the influence of NIMA exposure on the offspring T cell repertoire selection during ontogeny and on its $T_{cell}$ response during adulthood. First, we showed that adult NIMA mice were tolerant to Kβ as they accepted Kβ heart allografts permanently. This implies that these mice have been exposed to NIMA Kβ presumably during pregnancy or breastfeeding, or both. It is not clear whether this results from the transplacental passage of Kβ maternal cells or soluble Kβ molecules, or both. Several studies have documented the passage of hematopoietic maternal cells from the mother to the fetus (3, 23). Among them, T lymphocytes are regularly detected in umbilical cord blood samples from neonates. The presence of maternal T cells is commonly observed in SCID patients (24–33). A study by Kobayashi et al. (34) has documented that maternal CD4+ T cells are present in various tissues of a male infant with a SCID phenotype resulting from Artemis gene mutation. In a murine model system, involving the transfer of LacZ-, scid/scid, or wild type (+/+ ) blastocysts to pseudopregnant female animals, Piotrowski et al. (35) have demonstrated that in 90% of scid/scid fetuses, nucleated maternal cells were present in at least one lymphoid organ. In another study using GFP Tg female mice, Zhou et al. (36) have reported the presence of GFP+ maternal cells in fetal organs including the thymus, spleen, and liver. In addition, a recent study by Dutta et al. (37) demonstrates the presence of maternal hematopoietic microchimerism in lymphoid but also nonlymphoid organs, with predominance in the heart.

In this study, we showed that anti-Kβ TCR Tg CD8+ T cells present in the fetal and neonatal thymus of NIMA offspring display an activated phenotype. In addition, NIMA exposure is associated with a lower frequency of Kβ-specific T cells in the developing thymus of NIMA mice compared with control CBA (NE) mice. This suggests that from day 16.5 pc through the time of birth and up to 3 wk pp, the presence of NIMA was associated with either the deletion of some developing anti-Kβ T cells or an inefficient positive selection of these T cells. Unexpectedly, our results also show that thymocytes from the NIMA fetuses display a greater rate of proliferation while they are present at a lower frequency than their NE counterparts. The observation that TCR Tg developing thymic T cells from NIMA mice display a greater proliferation rate than those of NE mice support a partial deletional model rather than a lack of positive selection. Most important, the presence of normal frequencies of anti-Kβ T cells in adult NIMA mice demonstrates that tolerance to Kβ is not ensured only via deletion of anti-Kβ T cells during thymic development.

Functional analysis of anti-Kβ alloreactive T cells in NIMA mice revealed the absence of proinflammatory T cells producing IL-2 and IFN-γ. In turn, although these mice were tolerant to CBK allografts, they displayed some T cells producing IL-4 and high numbers of IL-10–producing T cells when challenged with Kβ allostimulators, that is, through the direct allorecognition pathway. Therefore, exposure of fetuses or neonates, or both, to NIMA resulted in the selection of T cells producing type 2 cytokines. Notably, the majority of the T cells producing IL-4 and IL-10 on stimulation with Kβ allogenic cells displayed a CD4+ phenotype. Indeed, the BM3.3 Tg mice used in this study were not bred on a RAG knockout background and displayed low but significant numbers of CD4+ T cells that were not Tg T cells (T98`). This suggested that, in NIMA mice, anti-Kβ CD8+ Tg T cells could not reject CBK allografts because they were suppressed by CD4+ T cells. This was confirmed by the observation that depletion of CD4+ T cells in NIMA mice restored their ability to reject CBK cardiac allografts. The presence of allospecific, IL-4–producing, CD4+ T cells in NIMA is consistent with the concept that neonatal tolerance is associated with activation of Th2 cells. In support of this, Fortshuber et al. have previously reported that neonatal tolerance is mediated via the positive selection of Th2 cells during development (38). Alternatively, it is possible that Tregs ensured tolerance to NIMA. Indeed, we showed that tolerance to Kβ could be transferred to control NE mice by injection of CD4+CD25+ Foxp3+ T cells collected from the spleens of NIMA mice. The majority of these tolerogenic CD4+ T cells secreted IL-10 and was donor-specific in that they did not suppress the rejection of third-party allografts (data not shown). Therefore, these Tregs are likely to correspond to inducible regulatory Tr1 cells rather than natural Tregs (39). This conclusion corroborates the results reported by others showing the presence of CD4+CD25+Foxp3+ Tregs secreting IL-10 and TGF-β in the lymph nodes of mice transplanted with a NIMA+ allograft (40–42). Further supporting this view, Mold et al. (43) have recently reported the presence of such Tregs in human fetal lymph nodes. Our study using adoptive transfer experiments demonstrates that these regulatory cells can be isolated and mediate allotransplant tolerance in vivo. This does not, however, exclude that some other cells, including CD8+ Tregs, and/or mechanisms can contribute to NIMA tolerance in this and other models.

The revelation of a powerful and beneficial NIMA effect in our Tg transplant model fully confirms and extends the original report of Owen et al. (44) regarding a tolerogenic effect of alloantigen pre-exposure on humoral immunity in adults. It is not clear why maternal chimerism and subsequent T cell tolerance to NIMA has been selected through evolution in mammals. It can be speculated that this process prevents the fetus’s immune system from attacking the mother as observed in GVH reactions. However, it seems unlikely that a few fetal T cells that are hyporesponsive to allostimulation could induce a life-threatening GVH-like disease in the mother. Alternatively, the passage of maternal leukocytes might be useful to protect the fetus against pathogens and/or contribute to its proper immune development and maturation. There are implications of the NIMA effect in pediatric transplantation where a child receives a kidney from his or her mother. In this setting, pretransplant maternal transfusion may reactivate and expand NIMA-specific Tregs, thereby amplifying the NIMA tolerogenic effect and ensuring tolerance to the transplant. The implications of the NIMA effect for a variety of other applications are numerous and include cord blood stem cell transplantation (45) and cadaveric organ transplantation, as well as nontransplant fields such as autoimmunity and development of antitumor vaccination approaches using “self” antigenic peptides, both of which may benefit from an understanding of the basic mechanisms of NIMA tolerance.

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Disclosures
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