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Prevention of Allogeneic Bone Marrow Graft Rejection by H-2 Transgene in Donor Mice

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Rejection of bone marrow grafts in irradiated mice is mediated by natural killer (NK) cells and is controlled by genes linked to the major histocompatibility complex (MHC). It has, however, not been possible to identify the genes or their products. An MHC class I (D^d) transgene introduced in C57BL donors prevented the rejection of their bone marrow by NK cells in irradiated allogeneic and F_1 hybrid mice expressing the D^d gene. Conversely, H-2D^d transgenic C57BL recipients acquired the ability to reject bone marrow from C57BL donors but not from H-2D^d transgenic C57BL donors. These results provide formal evidence that NK cells are part of a system capable of rejecting cells because they lack normal genes of the host type, in contrast to T cells, which recognize cells that contain abnormal or novel sequences of non-host type.

ACCEPTANCE AND REJECTION OF skin grafts can be predicted from the transplantation laws, reflecting the control of the T cell repertoire by genes for histocompatibility antigens (*1*). Only grafts expressing genes for antigens that are not expressed in the recipient can be recognized and eliminated by T cells. MHC-disparate bone marrow grafts can also be rejected by a T cell–independent mechanism involving NK cells (*2, 3*). With classical genetic crosses, it has not been possible to determine whether this type of rejection is triggered by expression of nonrecipient H-2, expression of foreign products encoded by other than the known MHC class I or class II genes, or absence of recipient H-2. We used transgenic mice to distinguish between these alternatives. Apart from their implications for basic immunology, the rules for rapid NK-mediated elimination of MHC-disparate hemopoietic cells are important for research on bone marrow transplantations, graft versus host reactions and viral diseases where transmission between individuals may occur by transfer of infected blood cells (*4*).

Transgenic mice of the D8 founder strain were produced by microinjection of an 8.0-kb genomic clone encoding H-2D^d and flanking regions from BALB/c into a C57BL/6 (B6) zygote (*5*). The cell surface expression of the H-2D^d transgene product showed the same tissue distribution and levels as the endogenous K^b and D^b products (*5*). A total of 321 mice of different genotypes received 900 rad of whole-body irradiation, and were then given either bone marrow from B6 or transgenic D8 mice, or no cells at all. Bone marrow engraftment was monitored by splenic ^{125}I-labeled iododeoxyuridine (^{125}IUdR) uptake on day 5. With this assay, it has been shown that B6 bone marrow is rejected by NK cells in H-2D^d–expressing B10.D2 and (B10.D2 × B6)F_1 mice (*2, 3*) (Fig. 1). If rejection was due to absence of H-2D^d products in the B6 marrow graft (*6, 7*), H-2D^d transgene expression in the donor should lead to acceptance, as was indeed observed (Fig. 1). The H-2D^d transgene had no effect on rejection of B6 marrow in B10.BR, an MHC disparate strain that does not carry the H-2D^d gene (Fig. 1).

These results are consistent with a model in which the transgene conferred protection selectively against a response, controlled by H-2D^d at the host level, that is geared to detect the absence of the corresponding gene or product in the graft (*6, 7*). According to this model, when D8 mice are recipients, expression of the H-2D^d transgene should be sufficient to render them capable of rejecting B6 marrow (even if they do not reject B6 skin grafts) (*8, 9*). Homozygous D8 as well as (D8 × B6)F_1 mice rejected B6 grafts, although the rejection in the F_1 hybrid appeared weaker (Fig. 1). H-2D^d transgenic bone marrow was accepted in both recipients.

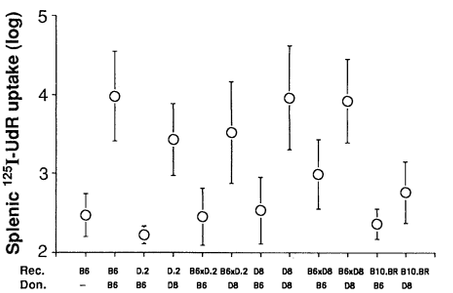

Fig. 1. Effect of H-2D^d transgene on engraftment of bone marrow in irradiated recipients (Rec.), monitored by ^{125}IUdR uptake in spleen. Bone marrow from tibia and femur was obtained by flushing with phosphate-buffered saline as described (*2*). One million bone marrow cells were grafted from B6 (H-2^b) and H-2D^d transgenic D8 donors (Don.) by intravenous inoculation to irradiated (900 rad) recipients of different genotypes. On day 5, 3 μCi of ^{125}IUdR (Amersham) was inoculated into mice intraperitoneally, and 18 to 24 hours later, the animals were killed and the radioactivity in the spleens was measured in an LKB gamma counter. After subtraction of background, $\log_{10}$ values were calculated. The geometric means and standard deviations in each group were calculated from several pooled experiments, each of which included several recipient genotypes in parallel. Each genotype was tested in two to four experiments, always with B6 and D8 grafts in parallel. Irradiated, nongrafted B6 mice and B6 mice grafted with syngeneic bone marrow were included in all experiments as controls. The incorporation of ^{125}IUdR in irradiated mice without grafts was similar in all genotypes and is shown here for B6 mice only. The difference between B6 and D8 donors was evaluated for each recipient by the Student's t test. ($P < 0.001$ in all cases, except in B10.BR recipients where the difference was nonsignificant.) D8, B6 (H-2^b), DBA/2 (H-2^d), and F_1 hybrids were bred and maintained at the Department of Tumor Biology. B10.D2 (D2, H-2^d) and B10.BR (H-2^k) were purchased from the Jackson Laboratory.

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Depletion of NK cells by treatment of recipients with monoclonal antibody to NK1.1 had a dramatic effect on engraftment of B6 marrow in H-2D^d–carrying congenic or transgenic mice (Fig. 2). The splenic ^{125}IUdR uptake reached the same levels as in mice given H-2D^d transgenic bone marrow, where the NK depletion had no significant effect. These results are in line with an earlier study showing that the responses observed with this protocol are T cell–independent and mediated by NK cells (*3*). We also observed an NK-dependent rejection of H-2D^d transgenic bone marrow in B6 recipients (Fig. 2). This rejection appeared weaker and more variable than the rejection in the opposite host-graft combination.

The equal engraftment in all donor-recipient combinations of mice treated with antibody to NK1.1 suggests that differences in the inherent colonization and growth potential of bone marrow stem cells cannot explain the selective growth of H-2D^d transgenic marrow in H-2D^d–expressing strains. Moreover, bone marrow of the two different genotypes showed similar engraftment in syngeneic recipients (Figs. 1 and 2) and similar formation of granulocyte-macrophage colony-forming units (GM-CFU) (*10*) in vitro (B6: 40.4 ± 13.2; D8: 50.4 ± 9.3; mean ± SD of two experiments).

The data thus show that introduction of an MHC sequence of recipient haplotype in the donor can rescue its otherwise H-2 allogeneic marrow from rejection, a result that is not predicted by the classical transplantation laws. The results do not exclude, but actually lend support to, the possibility that NK cell–dependent marrow rejection can be triggered also by the presence of a foreign MHC sequence in an otherwise syngeneic marrow (D8 graft to B6 recipient in Fig. 2). The rejection in the latter situation is seen also with transgenic skin grafts (*5, 8*) and is not controversial with respect to the T cell–based transplantation laws. Nevertheless, NK cells may contribute to rejection of bone marrow cells in such genetic combinations. Earlier studies showed that H-2D expression in the graft is not required to trigger rejection of MHC-disparate lymphoma cells in irradiated mice (*11, 12*), but did not exclude the possibility that rejection can be triggered either by the presence of foreign H-2 or by the absence of host H-2 sequence in the graft. It must be stressed that a different type of recognition mechanism may be operating in the situation where the transgene in the donor induced rejection rather than protection (Fig. 2), such as irradiation-resistant T cells or natural antibodies to H-2 triggering antibody-dependent cell-mediated cytotoxicity (*10*).

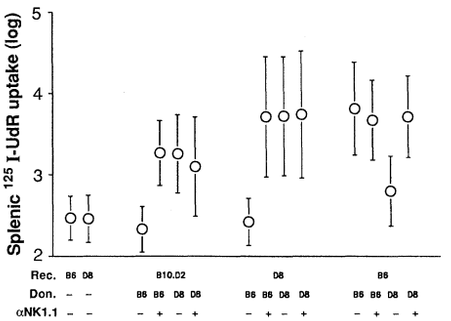

Fig. 2. Effect of NK depletion of recipients on engraftment of B6 and H-2D^d transgenic bone marrow. The experimental protocol was as described in Fig. 1, but recipients were treated with 200 μl of supernatant of an ascites-grown hybridoma PK 136, producing antibody to the allotypic NK1.1 marker (*21*) 1 day before bone marrow grafting. This treatment was sufficient to deplete animals of all detectable NK activity measured in a 4-hour ^{51}Cr-release test (*22*). Treatment with antibody to NK1.1 did not significantly affect the ^{125}IUdR uptake of irradiated control mice left without bone marrow infusion (*22*).

MHC-linked NK-mediated rejection of homozygous bone marrow grafts in allogeneic or F_1 hybrid mice is considered to reflect the action of recessive hemopoietic histocompatibility (Hh) genes rather than classical H-2K and D products (*12–16*). The Hh model and a role for MHC class I genes are not necessarily contradictory. First, our results do not exclude an independent role for structural or regulatory Hh genes mapping within the MHC in control of NK cells in marrow rejection. A second possibility is that Hh antigens are in fact controlled by MHC class I genes. The data positively identify a sequence containing the H-2D^d gene as an important factor at the donor level. Attempts to demonstrate transcripts from the flanking regions have failed (*17*). The most conservative interpretation leads to the conclusion that a part of the observed H-2D^d linkage of bone marrow engraftment in irradiated mice reflects a dominant effect of the H-2D^d gene itself. At the donor level, the mechanism may be a transacting downregulation of the structural gene for a putative target structure, as suggested by Rembecki *et al.* (*12*), or a masking of the latter at the cell surface level. Alternatively, the H-2D^d product may act as (or present) an inhibitory signal to NK cells of H-2D^d genotype. There are two main possibilities for the effect of the H-2D^d gene in the recipient. (i) It may define "self" in an "NK-educating" organ or tissue, or (ii) the critical expression may be in the NK cells themselves, where the transgene could exert a direct or indirect influence on target cell recognition. In any case, the effect of the transgene may not be restricted to the response against bone marrow cells; D8 mice are also relatively resistant to B6 lymphoma grafts (*18*).

In summary, our data provide evidence for three concepts of broad immunological interest. (i) Host-versus-graft responses involving well-defined MHC genes do not always follow the classical transplantation laws; (ii) NK cell–mediated rejection of bone marrow grafts depends on MHC class I genes of the donor; and (iii) the NK repertoire against normal cells is under influence of MHC class I genes in the host. The implication for physiological immunosurveillance of autologous cells is that loss or downregulation of normal MHC sequences (as in some transformed, undifferentiated or virus-infected cells) may trigger NK cell–mediated elimination (*7, 19*). As to rejection of MHC disparate cells, the irradiation-resistant NK reaction is relevant in bone marrow transplantation (*2, 3, 12–16*) and graft versus host disease (*20*). A rapid defense against small numbers of cells from another individual may be considered nonphysiological according to the immunological textbook cliché. However, such transfer of cells can occur during sexual contacts and pregnancy, and may be a significant mechanism for spread of certain virus infections such as human immunodeficiency virus (HIV) (*4*).

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Vaccination Against Experimental Allergic Encephalomyelitis with T Cell Receptor Peptides

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system mediated by CD4$^+$ T cells reactive with myelin basic protein (MBP). Rats were rendered resistant to the induction of EAE by vaccination with synthetic peptides corresponding to idiotypic determinants of the β chain VDJ region and Jα regions of the T cell receptor (TCR) that are conserved among encephalitogenic T cells. These findings demonstrate the utility of TCR peptide vaccination for modulating the activity of autoreactive T cells and represent a general therapeutic approach for T cell–mediated pathogenesis.

ENCEPHALITOGENIC T CELLS (*1–3*) from mouse and rat models of EAE use common germline TCR genes (*4–8*), despite known differences in MBP-peptide specificity and presumed differences in major histocompatibility complex (MHC) restriction [reviewed in (*9*)]. The Vβ8.2 genes are preferentially utilized by encephalitogenic T cells in PL/J, (PL/J × SJL)F_1 (*5*), and B10.PL (*6*) mice. TCR α chain gene usage is similarly restricted, with Vα4 exclusively used in PL/J and (PL/J × SJL)F_1 mice and Vα2 and Vα4 used equally in B10.PL mice. In B10.PL mice, these α chain V region genes are rearranged only to the JαTA39 gene, while JαTA31 is primarily used in PL/J mice. This restricted TCR gene usage is consistent with the common MBP peptide specificity (acetylated residues 1 to 11) and MHC restriction (Ia^u) of encephalitogenic T cells in these mouse models (*6, 10*). Encephalitogenic T cells from Lewis rats also show restricted TCR gene usage, in accord with their common specificity for MBP residues 68 to 88 in the context of the rat RT-1^b MHC haplotype (*11*). TCR gene usage in Lewis rat EAE is identical to that in the mouse models described above. Vβ8.2 is used in conjunction with Vα2 that has been rearranged to JαTA39. Common TCR gene usage among mouse and rat encephalitogenic T cells is enigmatic, because different, noncrossreactive combinations of MHC and peptide antigen (*9*) are recognized in the respective models. The EAE induced by T cells in rats or mice is histologically identical, which supports a role for the reported TCRs in the pathogenesis of EAE.

To define EAE-associated idiotypic determinants, we analyzed published sequences of TCR genes from encephalitogenic T cells in mouse and rat models of EAE. Amino acid sequence was conserved in the region of TCR β chain VDJ joining and in the Jα elements of these T cells (Fig. 1). The B10.PL sequence (Fig. 1A) is that reported for four of five encephalitogenic T cell clones whose Vβ8 rearrangements were sequenced (*6*). Similar β chain VDJ sequences are reported for three independently isolated Lewis rat Vβ8 T cell clones (*7, 8*). In PL/J and (PL/J × SJL)F_1 mice, four different TCR β chain VDJ sequences were reported (*5*); only one had limited homology with these sequences.

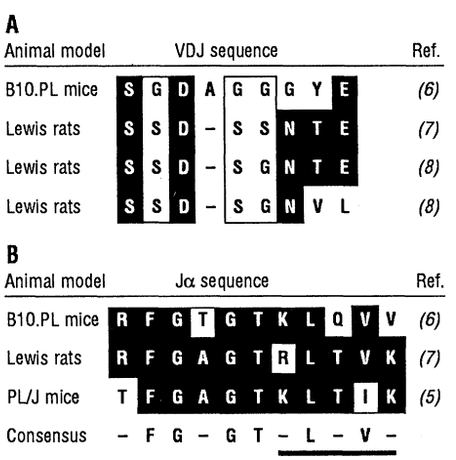

Fig. 1. T cell receptor β chain VDJ (**A**) and Jα (**B**) sequences in EAE. Literal homologies are boxed and shaded; substitutions acceptable according to a Dayhoff matrix are boxed but unshaded. The 75% consensus mouse Jα residues were taken from (*12*). The bold line in (B) denotes r-JαTA39 residues conforming to patterns characteristic of T cell epitopes.

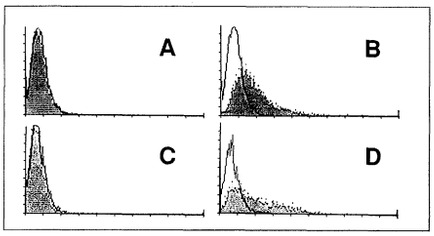

Fig. 2. Cytofluorometry of Lewis rat MBP-reactive T cells stained with antibodies to TCR peptides. A Lewis rat MBP-reactive T cell line in its fifth in vitro cycle of MBP stimulation (approximately one-third Vβ8$^+$) was incubated with protein A–purified immunoglobulin G (IgG) from pre-immune sera (**A**), affinity purified Abs to r-VDJ2 (**B**), pre-immune IgG (**C**), or affinity-purified Abs to JαTA39 (**D**). Antibody binding was revealed with fluorescein-conjugated goat Abs to rabbit IgG. Specific binding is indicated by shaded profiles and background staining of goat Abs to rabbit Ig by unshaded profiles.

The JαTA39 sequences used in B10.PL mice (*6*) and Lewis rats (*7*) (Fig. 1B) display homology at 9 of the 11 residues shown. The JαTA31 sequence from PL/J mice (*5*) shares ten residues with the Lewis rat JαTA39 sequence and eight residues with the B10.PL mouse JαTA39 sequence. Analysis of 27 additional mouse Jα sequences (*12*) identified only one other very similar sequence, JαTA37, which is consistent with the restricted Jα gene usage in EAE. The conservation of β chain VDJ and Jα sequences among encephalitogenic T cells, even in different animal models of EAE, implicates these TCR idiotopes in the pathogenesis of EAE and provides targets for immunotherapeutic intervention.

We vaccinated Lewis rats with synthetic peptides corresponding to these TCR idiotopes (Table 1). In initial experiments, the m-VDJ peptide was administered in complete Freund's adjuvant (CFA). Age- and gender-matched control rats received either CFA alone or were not vaccinated. Thirty days after vaccination, all animals were challenged with guinea pig MBP in CFA. Disease, characterized by severe paralysis and wasting, was observed in unvaccinated control animals by day 10 or 11 (Table 2), persisted for 5 to 6 days, and spontaneously remitted. Disease courses among CFA-vaccinated rats were not significantly different from unvaccinated controls. In contrast, disease in the rats vaccinated with m-VDJ (5,

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