Tcrb-V3⁺ T-cell deletion and a mouse mammary tumor provirus, *Mtv-27*

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**Abstract.** Genes encoding superantigens which delete Tcrb-V3⁺ T cells co-segregate with mouse mammary tumor proviruses (*Mtv*), *Mtv-1*, *Mtv-3*, *Mtv-6*, *Mtv-13*, and *Mtv-44*. We have examined percentages of Tcrb-V3⁺ T cells and *Mtv* integrations in ([B10 × NZB]F1 × B10.BR) mice, and show that *Mtv-27* as well as *Mtv-3* from NZB mice co-segregate with genes encoding deletion ligands for Tcrb-V3⁺ T cells without recombination.

**Introduction**

Superantigens in association with major histocompatibility complex (MHC) class II molecules delete certain Tcrb-V-bearing T cells at a CD4⁺CD8⁺ stage of differentiation in the thymus (Kappler et al. 1987, 1988; MacDonald et al. 1988). These antigens are thought to be encoded by mouse mammary tumor viruses (MMTV): genes encoding endogenous superantigens co-segregate with mouse mammary tumor provirus (*Mtv*) genomes without recombination (Woodland et al. 1990, 1991a; Frankel et al. 1991; Tomonari and Fairchild 1992).

Furthermore, *Orf* genes in the 3' long terminal repeat sequence (LTR) of *Mtv* and exogenous MMTV have been shown to confer superantigen activity on the transfected cells and in the transgenic mice (Choi et al. 1991; Acha-Orbea et al. 1991; Woodland et al. 1991b; Pullen et al. 1988). It has been shown that superantigens encoded by these viral genomes specifically delete certain Tcrb-V-bearing T cells: *Mtv-1*, *Mtv-3*, *Mtv-6*, *Mtv-13*, and *Mtv-44* for Tcrb-V3⁺ T cells (Frankel et al. 1991; Fairchild et al. 1991, 1992), *Mtv-6* and *Mtv-9* for Tcrb-V5⁺ T cells (Woodland et al. 1990, 1991a; Acha-Orbea and Palmer 1991), *Mtv-7* and *Mtv-44* for Tcrb-V6⁺, Tcrb-V8.1⁺, and Tcrb-V9⁺ T cells (Frankel et al. 1991; Tomonari and Fairchild 1992), *Mtv-7* for Tcrb-V7⁺ T cells (Frankel et al. 1991), *Mtv-8*, *Mtv-9*, and *Mtv-11* for Tcrb-V11⁺ T cells (Dyson et al. 1991; Woodland et al. 1991a), and MMTVs for Tcrb-V14⁺ T cells (Marrack et al. 1991; Choi et al. 1991; Acha-Orbea et al. 1991). In this paper we examined segregation of *Mtv* genomes and genes encoding deletion ligands for Tcrb-V3⁺ T cells in ([B10 × NZB]F1 × B10.BR) mice.

**Materials and methods**

*Mice.* C57BL/10 (B10) and B10.BR mice were obtained from the Clinical Research Centre (Harrow, UK). NZB mice were obtained from Ouac (Bicester, UK). ([B10 × NZB]F1 × B10.BR) mice were produced in the Clinical Research Centre.

**Analysis with a fluorescence-activated cell sorter (FACS).** 1.2 × 10⁶ mesenteric lymph node cells were stained as described previously (Tomonari and Fairchild 1991). First step reagents were fluorescein isothiocyanate (FITC)-coupled antibodies, KT15 (anti-CD8c⁺; Tomonari and Spencer 1990) and KT6 (anti-CD4; Tomonari and Fairchild 1991), and biotinylated antibody KJ25 (anti-V33; Pullen et al. 1988). Streptavidin phycoerythrin (Biogenesis, Bournemouth, UK) was used as a second step reagent. 5 × 10⁴ cells were examined by two-color FACS analysis (FACStarPLUS, Becton-Dickinson, Mountain View, CA).

**Southern blot analysis.** 15 µg of DNA, prepared as described previously (Maniatis et al. 1982), was digested with EcoRI (Gibeo BRL, Uxbridge, UK) and restricted fragments were separated through 0.7% agarose and transferred to nylon membranes (Genescreen, DuPont, Boston, MA). An *Mtv* LTR probe, generated from the 3' end of *Mtv*-9 with Bgl II and Msp I, was labeled with random oligo primed α³²PdCTP to 10⁸ cpm/µg. Following overnight hybridization with the probe at 65°C, filters were washed at high stringency [0.1 x standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS)] for 90 min at 65°C, dried and exposed for 24 h to Kodak X-ray film with intensifying screens.

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Results

Two genes are responsible for Tcrb-V3+ T-cell deletion in NZB mice. NZB and (B10 × NZB)F1 hybrid mice delete Tcrb-V3+ T cells (0.1%–0.2%), whereas B10 and B10.BR mice do not (3.1%–5.0%). To examine the number of genes responsible for the deletion in NZB mice, (B10 × NZB)F1 hybrid mice were crossed with B10.BR mice. Percentages of peripheral Tcrb-V3+ T cells from [B10 × NZB]F1 × B10.BR mice were then examined by two-color FACS analysis (Fig. 1). Of the 84 offspring, 17 mice (20%) did not delete Tcrb-V3+ T cells (2.6%–4.7%), whereas 67 mice (80%) completely deleted these T cells (0.0%–0.2%). This ratio suggests that two genes independently encode deletion ligands for these T cells.

Co-segregation of genes encoding deletion ligands for Tcrb-V3+ T cells with Mtv-3, Mtv-7, and/or Mtv-27. Because a number of Mtv genomes co-segregate with genes encoding deletion ligands for Tcrb-V3+, Tcrb-V5+, Tcrb-V6+, Tcrb-V7+, Tcrb-V8.1+, Tcrb-V9+, and Tcrb-V11+ T cells in various inbred mice (Woodland et al. 1990, 1991a; Frankel et al. 1991; Dyson et al. 1991; Fairchild et al. 1991, 1992; Pullen et al. 1992; Tomonari and Fairchild et al. 1992), Mtv integration was examined in DNA from the 84 [(B10 × NZB)F1 × B10.BR] mice by Southern blot analysis using the Mtv LTR probe. Representative blots from 41 mice (27 deletor and 14 nondeleter) are shown in Figure 2. NZB mice have Mtv-3 [(19.3 and 6.7 kilobases (kb)], Mtv-7 (16.5 and 12.0 kb), Mtv-9 (9.7 and 7.4 kb) Mtv-14 (1.7 kb, not shown in Figure 2), Mtv-17 (9.9 and 7.9 kb), Mtv-27 (11.4 kb) and Mtv-28 (5.8 kb). B10 and B10.BR mice have Mtv-8 (7.7 and 6.3 kb), Mtv-9 (9.7 and 7.4 kb), and Mtv-17 (9.9 and 7.9 kb). The 67 [(B10 × NZB)F1 × B10.BR] mice which completely deleted Tcrb-V3+ T cells inherited Mtv-3, Mtv-7, and/or Mtv-27. Although Mtv-7 is closely linked to Mtv-27 on chromosome 1 (Eicher and Lee 1990), there was one recombinant offspring (with Tcrb-V3+ T-cell deletion in the absence of Mtv-3) which inherited Mtv-27 but not Mtv-7 (arrow in Figure 2). Neither Mtv-3, Mtv-7, nor Mtv-27 was inherited by the 17 offspring which did not delete these T cells.

Discussion

The above data suggest that Mtv-3 and Mtv-27 in NZB mice encode deletion ligands for Tcrb-V3+ T cells. Although Mtv-7 co-segregated with Mtv-27 and a gene encoding the deletion ligand, it is likely that Mtv-27 but not Mtv-7 encodes the deletion ligand. The reasons are twofold. First, there was a recombinant Mtv-7 Mtv-27+ mouse which deleted Tcrb-V3+ T cells in the absence of Mtv-3. Secondly, in other strains of mice Mtv-7 co-segregates with a gene encoding a deletion ligand for Tcrb-V6+ T cells but not for Tcrb-V3+ T cells (Frankel et al. 1991; Lee and Eicher 1990; our unpublished data). Because we have not yet found any Mtv-3 ‘Mtv-7+ Mtv-27-’ [(B10 × NZB)F1 × B10.BR] mouse, the possibility that Mtv-7 of NZB mice encodes a deletion ligand for Tcrb-V3+ T cells remains to be determined. Mtv-27 is thought to be located 7 centimorgan downstream from Mtv-7 on chromosome 1 (Hillyard et al. 1991). However, this distance appears to be much shorter as we found only one recombinant offspring (Mtv-7 ‘Mtv-27+’) out of 84 [(B10 × NZB)F1 × B10.BR] mice (Fig. 2).

Mtv-27 is a sixth Mtv genome which deletes Tcrb-V3+ T cells in addition to Mtv-6, Mtv-13, Mtv-3, Mtv-1, and Mtv-44 which have previously been shown to delete these T cells (Frankel et al. 1991; Fairchild et al. 1991, 1992; Pullen et al. 1992). Of these, Mtv-6 and Mtv-44 also delete T cells bearing other Tcrb-V elements: Tcrb-V5+ T cells by Mtv-6 (Acha-Orbea and Palmer 1991) and Tcrb-V6+, Tcrb-V8.1+, and Tcrb-V9+ T cells by Mtv-44 (Tomonari and Fairchild 1992). If the specificity for Tcrb-V elements is determined by the carboxy terminal region of Orf proteins as proposed (Choi et al. 1991; Pullen et al. 1992), then the proteins encoded by Mtv-1, Mtv-3, Mtv-13, and possibly by Mtv-27 and Mtv-44 would also be expected to delete Tcrb-V5+ T cells because amino acid sequences of these former three proteins and the Mtv-6 Orf are the same or nearly identical (Pullen et al. 1992). Similarly, Orf proteins encoded by Mtv-8 and Mtv-11 would be expected to delete Tcrb-V5+ T cells, because the Mtv-9 Orf, which is very similar to these in amino acid sequence (Choi et al. 1991), has been demonstrated to delete Tcrb-V5+ T cells (Woodland et al. 1990, 1991a). Furthermore, the Mtv-8 Orf has been