*Brief Definitive Report*

# CD4 Expression Is Differentially Required for Deletion of MLS-1$^a$-reactive T Cells

By Valerie A. Wallace, Amin Rahemtulla, Emma Timms, Josef Penninger, and Tak W. Mak

*From the Ontario Cancer Institute, Department of Medical Biophysics and Immunology, University of Toronto, Toronto, Ontario M4X 1K9, Canada*

## Summary

Clonal deletion of thymocytes expressing potentially self-reactive T cell receptors (TCRs) occurs during thymocyte ontogeny. Mice deficient for CD4 expression provide a unique model system to study the contribution of the CD4 molecule in negative selection of T cells reactive against the major histocompatibility complex class II–associated retroviral self-superantigen, Mls-1$^a$. In the presence of Mls-1$^a$ determinants, mature CD8$^+$ T cells expressing V$\beta$6, 8.1, and 9 were deleted in CD4-deficient mice, thus demonstrating that TCR affinity for Mls-1$^a$ is sufficient for deletion and that a signal through CD4 was not required. However, in instances where the TCR affinity for Mls-1$^a$ is low, as in the case of V$\beta$7$^+$ T cells, CD4 expression was required for clonal deletion. These results demonstrate that for Mls-1$^a$-mediated clonal deletion of T cells, the requirement for the accessory or coreceptor function of CD4 depends on the affinity of the TCR.

One mechanism by which self-tolerance is maintained is through clonal deletion of T cells expressing self-reactive TCRs during thymocyte ontogeny (1). The nature of the tolerizing signal delivered to the developing thymocyte, however, has not yet been characterized. In addition, the contribution of other cell surface molecules, for instance, CD4, to tolerance induction is still controversial.

Recently, it has been demonstrated that Mls-1$^a$ determinants are encoded by an endogenous mouse mammary tumor virus, Mtv-7, integrated on chromosome 1 (2, 3). T cells expressing V$\beta$6, 7, 8.1, and 9 are deleted during thymic differentiation in Mls-1$^a$ strains of mice (4). Mls-1$^a$ stimulation in vitro of T cells or clonal deletion of thymocytes requires the presence of MHC class II molecules, and there is a hierarchy for MHC class II presentation of Mls-1$^a$; namely, H-2$^k$, H-2$^d$ > H-2$^b$ >> H-2$^q$, with I-E molecules being better presenters than I-A molecules (4).

Mice rendered CD4 deficient by gene targeting (5) provide a unique system in which to study the requirement for CD4 in negative selection of CD8$^+$ T cells mediated by self-superantigens. TCR V$\beta$ expression was examined in CD4-deficient mice (CD4$^{-/-}$) bred into Mls-1$^a$ and Mls$^b$ backgrounds. V$\beta$6$^+$, 8.1$^+$, and 9$^+$ T cells were deleted in CD4$^{-/-}$ Mls-1$^a$ mice in I-E$^+$ haplotypes. However, V$\beta$7$^+$ T cells were not deleted in CD4$^{-/-}$ Mls-1$^a$ mice.

## Materials and Methods

*Mice and Mtv-7 Analysis.* B10.BR, BALB.K, CBA/J, and DBA/2 strains of mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice homozygous for the CD4 mutation (CD4$^{-/-}$) have been described previously (5). The breeding strategy to obtain CD4$^{-/-}$ Mls-1$^a$ mice was as follows: CD4 heterozygous (CD4$^{+/-}$) $F_1$ mice were obtained from crosses between female DBA/2 (H-2$^d$, Mls-1$^a$), CBA/J (H-2$^k$, Mls-1$^a$), or B10.BR (H-2$^k$, Mls$^b$), and male CD4$^{-/-}$ H-2$^b$ Mls$^b$ animals. The CD4$^{+/-}$ $F_1$ progeny from each pair were mated, and pups from the $F_2$ generation were typed for CD4 expression and H-2 haplotype by immunofluorescence. Mice were typed for Mls-1$^a$ by Southern blot analysis for Mtv-7 integration, which is determined by the presence of an 11.7-kb band in blots of EcoRI-digested genomic DNA probed with an MMTV env probe (6, 7, and data not shown).

*In Vitro Culture.* Spleen cell responders (2 × 10$^6$) from mice aged 6–12 wk were cocultured with 5 × 10$^6$ anti-Thy-1 and complement-depleted and irradiated (1,000 rad) splenic stimulator cells in 2 ml of IMDM supplemented with 10% FCS, L-glutamine, 2-ME, antibiotics, and 10% rat spleen Con A supernatant as a source of IL-2. In experiment 2, responder cells (2 × 10$^6$) were stimulated with 2.5 $\mu$g/ml Con A (Pharmacia, Uppsala, Sweden) in medium lacking IL-2. On day 3, responding T cell blasts were isolated on a Ficoll density gradient and cultured for another 2 d in the presence of IL-2, after which the cells were harvested and stained for CD8 and TCR V$\beta$ expression.

*Immunofluorescence.* Blood samples (200 $\mu$l) were collected in heparinized capillary tubes, washed once in immunofluorescence staining buffer (PBS, 4% FCS, 0.1% $NaN_3$), and incubated with the following anti-H-2 culture supernatants obtained from American Type Culture Collection (Rockville, MD): B8-24 (anti-H-2$^b$) (TIB 139), 34-2-12 (anti-H-2$^d$) (HB87), and 11-4-1 (anti-H-2$^k$) (TIB 95), followed by FITC-conjugated goat anti-mouse antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL). For CD4 analysis, blood samples were stained with PE-conjugated anti-CD4 (Becton Dickinson & Co., Mountain View, CA). For TCR

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/11/1459/05 $2.00
Volume 176 November 1992 1459–1463

Vβ analysis, the following rat mAb cell culture supernatants were used: 44-22-1 (anti-Vβ6) (8), TR31 (anti-Vβ7) (9), KJ16 (anti-Vβ8.1, 8.2) (10), and B20.6 (anti-Vβ2) (11). The following mouse mAbs were used: F23.2 (anti-Vβ8.2) (12) and MR10-2 (anti-Vβ9) (13). Single cell suspensions of thymocytes and mesenteric lymph nodes from 6–8-wk-old mice were resuspended in staining buffer and incubated with appropriate anti-TCR Vβ culture supernatants, washed, and labeled with PE-conjugated goat anti-rat Ig or FITC-conjugated goat anti-mouse Ig (Southern Biotechnology Associates, Inc.). Lymph node samples were incubated with 1 μg of rat IgG (Sigma Chemical Co., St. Louis, MO) to block remaining anti-rat Ig sites, and cells were then double stained with anti-CD8 biotin (Becton Dickinson & Co.) followed by Streptavidin-RED613 (Gibco Laboratories, Grand Island, NY). T cell blasts were stained as described for lymph node samples. Samples were analyzed using a FACScan® (Becton Dickinson & Co.). The percentage of $V\beta8.1^+$ T cells was calculated by subtracting the percentage of $V\beta8.2^+$ cells from the percentage of $V\beta8.1^+$ + $V\beta8.2^+$ cells. Mean values of TCR Vβ expression were statistically compared between Mls-$1^a$ and $Mls^b$ mice within the same H-2 haplotype using an unpaired Student's *t* test. Probability values, $p < 0.05$, were taken to be significantly different.

## Results and Discussion

To assess the requirement for CD4 expression in the Mls-$1^a$-mediated deletion of $CD8^+$ T cells bearing Vβ6, 7, 8.1, and 9, the CD4 mutation was bred into different Mls and H-2 backgrounds, as described in Materials and Methods. The presence of Mls-$1^a$ was determined by Southern blot analysis for Mtv-7 integration (data not shown). We first determined whether the lack of CD4 expression during ontogeny would adversely affect the development of Mls-$1^a$-reactive T cells in CD4 deficient ($CD4^{-/-}$) $Mls^b$ ($Mtv\text{-}7^-$) mice. As previously reported, $CD4^{-/-}$ mice have a large peripheral TCR $\alpha/\beta^+$ $CD8^+$ T cell population and a smaller TCR $\alpha/\beta^+$ $CD4^-CD8^-$ population (5). $CD8^+$ peripheral T cells expressing Vβ6, 7, 8, 1, and 9 were present in the lymph nodes of $CD4^{-/-}$ $Mls^b$ H-$2^k$ or H-$2^d$ mice at levels similar to $CD4^{+/-}$ $Mls^b$ littermates or BALB.K ($Mls^b$, H-$2^k$) mice (Table 1 and Fig. 1).

After in vitro Mls-$1^a$ stimulation, $CD8^+$ T cell blasts from $CD4^{-/-}$ $Mls^b$ mice were enriched for Vβ6 and 8.1 expression, as were $CD8^+$ T blasts from control BALB.K mice (Table 2). $CD8^+$ T cells expressing $V\beta7^+$ TCRs were poorly enriched in anti-Mls-$1^a$ cultures from both the $CD4^{-/-}$ $Mls^b$ or control BALB.K mice, which is in agreement with previous data showing that $V\beta7^+$ T cells are the least Mls-$1^a$ reactive in vitro (14). $V\beta9^+$ T cells were also not significantly enriched in these cultures, and this may be due to the strain combination used in this experiment. Therefore, the lack of CD4 expression during thymic ontogeny in $CD4^{-/-}$ $Mls^b$ mice did not affect the development of T cells expressing Mls-$1^a$-reactive Vβs or in vitro reactivity against Mls-$1^a$ determinants.

Spleen cells from $CD4^{-/-}$ Mls-$1^a$ H-$2^d$ or H-$2^k$ mice were tolerant against Mls-$1^a$ as judged by low cell recovery and lack of enrichment for Vβ6, 7, 8.1, or 9 $CD8^+$ T cells after a 5-d in vitro Mls-$1^a$ stimulation (Table 2, and data not shown). Lymph node cells from $CD4^{-/-}$ Mls-$1^a$ mice were analyzed for expression of Mls-$1^a$-associated Vβs in order to determine if clonal deletion was the mechanism of tolerance against Mls-$1^a$ (Table 1, and Fig. 1). $CD8^+$ $V\beta6^+$ T cells were significantly reduced in $CD4^{-/-}$ Mls-$1^a$ H-$2^d$ and H-$2^k$ mice when compared with their $CD4^{-/-}$ $Mls^b$ littermates ($p < 0.05$). $V\beta6^+$ T cells were also not present in the $CD4^-CD8^-$ compartment of $CD4^{-/-}$ Mls-$1^a$ mice (data not shown). $CD8^+$ T cells bearing Vβ8.1 and 9 were also reduced in $CD4^{-/-}$ Mls-$1^a$ H-$2^d$ mice to the same extent as in $CD4^+$ Mls-$1^a$ H-$2^d$ littermates and CBA/J (H-$2^k$, Mls-$1^a$) control mice. These results demonstrate that in the absence of CD4 expression, $CD8^+$ T cells bearing Vβ6, 8.1, and 9 can be deleted by Mls-$1^a$ determinants.

**Table 1.** *TCR Vβ Expression on $CD8^+$ Lymph Node T Cells from Control and $CD4^{-/-}$ Mice*

| Mouse* | Mls | H-2 | I-E | Percent of $CD8^+$ lymph node cells expressing: Vβ6 | Vβ7 | Vβ8.1 | Vβ9 | Vβ2‡ |
|---|---|---|---|---|---|---|---|---|
| BALB.K | b | k | + | 11.6 ± 1.0 | 3.2 ± 0.5 | 15.8 ± 1.7 | 2.1 ± 0.3 | 6.7 ± 2.0 |
| CBA/J | a | k | + | 0.1 ± 0.0 | 0.9 ± 0.5 | 5.5 ± 1.2 | 0.2 ± 0.1 | 16.5 ± 1.2 |
| $CD4^{+/-}$ | b | d | + | 12.1 ± 0.3 | 4.0 ± 1.8 | 11.4 ± 3.3 | 3.1 ± 0.9 | 7.7 ± 1.4 |
| $CD4^{+/-}$ | a | d | + | 0.6 ± 0.1 | 1.3 ± 0.3 | 3.3 ± 0.5 | 0.6 ± 0.3 | 8.7 ± 0.7 |
| $CD4^{-/-}$ | b | d | + | 10.6 ± 0.7 | 5.0 ± 1.6 | 9.2 ± 1.6 | 2.0 ± 0.1 | 6.3 ± 1.1 |
| $CD4^{-/-}$ | a | d | + | 1.4 ± 0.2 | 2.9 ± 0.5 | 5.5 ± 0.1 | 0.9 ± 0.1 | 6.6 ± 0.5 |
| $CD4^{-/-}$ | b | k | + | 8.9 ± 2.0 | 4.5 ± 0.6 | ND | ND | 7.0 ± 1.0 |
| $CD4^{-/-}$ | a | k | + | 1.4 ± 0.2 | 3.2 ± 0.4 | ND | ND | 9.9 ± 1.0 |

Mice were typed for CD4, H-2, and Mls-$1^a$ as described in Materials and Methods. Mesenteric lymph node cell suspensions from mice aged 6–8 wk were stained with appropriate anti-TCR Vβ mAbs and anti-CD8, and analyzed by flow cytometry. Results are expressed as mean percent of $V\beta^+$ $CD8^+$ T cells ± SEM. Underlined values are not significantly different from one another.

* At least three mice per group were analyzed.

‡ Staining for Vβ2, which is not Mls-$1^a$ reactive, is also included.

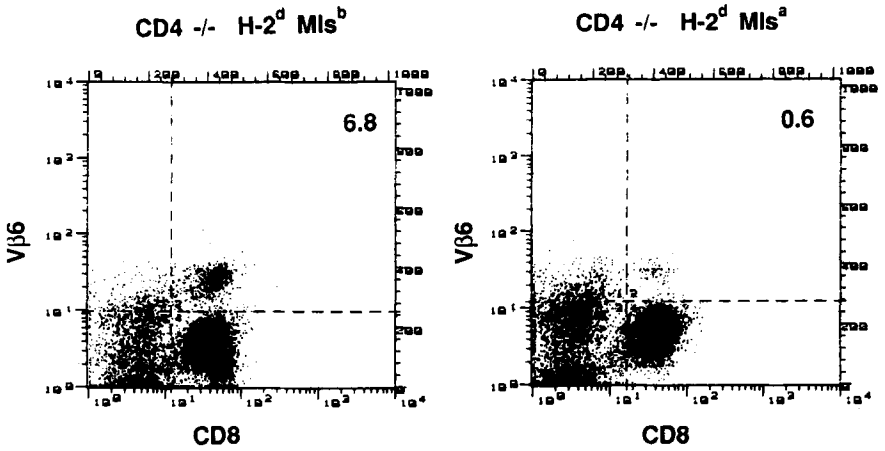


**Figure 1.** Vβ6 expression on CD8+ T cells from lymph nodes of CD4$^{-/-}$ mice. Cell suspensions of mesenteric lymph nodes from 6–8-wk-old CD4$^{-/-}$ H-2$^d$ Mls$^b$ and Mls-1$^a$ mice were double stained for Vβ6 and CD8 expression, as described in Materials and Methods.

T cells expressing Vβ7 were not significantly reduced in the lymph nodes of CD4$^{-/-}$ Mls-1$^a$ H-2$^d$ or H-2$^k$ mice as compared with CD4$^{+/-}$ Mls-1$^a$ H-2$^d$ or H-2$^k$ littermates, or CBA/J mice, thus demonstrating that CD4 expression is required for deletion of Vβ7-bearing T cells (Table 1, and data not shown). Although mice heterozygous for the CD4 mutation (CD4$^{+/-}$) have a slightly reduced intensity of surface CD4 staining (5), Vβ7$^+$ T cells were deleted in CD4$^{+/-}$ Mls-1$^a$ H-2$^d$ mice to the same extent as in CBA/J mice (Table 1). Therefore, a reduction in CD4 surface intensity on T cells does not adversely affect deletion of Vβ7$^+$ T cells.

We next investigated whether deletion of Vβ6$^+$ T cells could be detected in the thymus of CD4$^{-/-}$ Mls-1$^a$ mice. In Mls-1$^a$ mouse strains, T cells expressing Mls-1$^a$-reactive Vβs are present among the TCR$^{lo}$ immature thymocyte population, but are markedly reduced in the mature TCR$^{hi}$ thymocyte population (1). As summarized in Table 3, the Vβ6$^{hi}$ population of thymocytes was present in CD4$^{-/-}$ Mls$^b$ and in control BALB.K (Mls$^b$, H-2$^k$) mice. However,

**Table 2.** *TCR Vβ Expression on CD8+ T Cell Blasts after In Vitro Mls-1$^a$ Stimulation*

| Exp. | Responder | Stimulator | Percent of CD8+ T cell blasts | | | | |
|---|---|---|---|---|---|---|---|
| | | | Vβ6 | Vβ8.1 | Vβ8.2 | Vβ7 | Vβ9 |
| 1 | BALB.K | BALB.K | 7.1 | NT* | NT | NT | NT |
| | H-2$^k$, Mls$^b$ | CBA/J‡ | 43.0 | 14.6 | 3.3 | NT | NT |
| | CD4$^{-/-}$ | BALB.K | 7.7 | 9.7 | 14.1 | NT | NT |
| | H-2$^k$, Mls$^b$ | CBA/J | 41.5 | 17.0 | 7.6 | NT | NT |
| | CD4$^{-/-}$ | BALB.K | 2.9 | NT | NT | NT | NT |
| | H-2$^k$, Mls-1$^a$ | CBA/J | 2.1 | 5.6 | 14.4 | NT | NT |
| 2 | BALB.K | Con A | 13.1 | 18.4 | 16.2 | 10.2 | 3.3 |
| | | BALB.K | 16.5 | NT | NT | NT | NT |
| | | CBA/J | 66.0 | 17.2 | 3.7 | 15.0 | 2.4 |
| | CD4$^{-/-}$ | Con A | 12.4 | 11.4 | 19.2 | 5.0 | 3.1 |
| | H-2$^k$, Mls$^b$ | BALB.K | 10.8 | 10.9 | 17.0 | 6.8 | 3.5 |
| | | CBA/J | 64.0 | 11.1 | 13.3 | 2.8 | 1.5 |
| | CD4$^{-/-}$ | Con A | 1.1 | 0.3 | 27.4 | 4.0 | 1.3 |
| | H-2$^k$, Mls-1$^a$ | BALB.K | 1.4 | 3.8 | 20.3 | 3.9 | NT |
| | | CBA/J | 0.9 | 7.9 | 16.0 | 2.8 | 1.3 |

Spleen cell responders (2 × 10$^6$) from mice aged 6–12 wk were stimulated as described (Materials and Methods) and double stained with anti-Vβ mAb and CD8.

* NT, not tested.

‡ CBA/J stimulators are H-2$^k$, Mls-1$^a$.

**Table 3.** *TCR $V\beta^{high}$ Expression on Thymoctyes from $CD4^{-/-}$ Mice*

| Mice | Mls | H-2 | Percent of Vβ high-intensity thymocytes | | |
|---|---|---|---|---|---|
| | | | Vβ6 | Vβ7 | Vβ2 |
| BALB.K | b | k | 1.1 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 |
| DBA/2 | a | d | 0.4 ± 0.1 | 0.2 ± 0.0 | 0.5 ± 0.0 |
| $CD4^{-/-}$ | b | k | 1.6 ± 0.3 | 0.6 ± 0.1 | 0.9 ± 0.2 |
| $CD4^{-/-}$ | a | k | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.9 ± 0.3 |
| $CD4^{-/-}$ | a | d | 0.3 ± 0.1 | 0.4 ± 0.2 | 0.7 ± 0.4 |

Single cell suspensions of thymocytes from 6–8-wk-old mice were stained for Vβ6, Vβ7, and Vβ2 expression. Samples were analyzed on a FACScan® and the percentage of thymocytes with high-intensity TCR Vβ expression was determined. Results are expressed as mean percent of $V\beta^{high}$ thymocytes ± SEM, and the data are compiled from at least three animals per group. It should be noted that the percentages of TCR $\alpha/\beta^{high}$ thymocytes were similar in all mice tested.

the $V\beta6^{hi}$ population was greatly reduced in thymus samples from $CD4^{-/-}$ Mls-$1^a$ and control DBA/2 (Mls-$1^a$, H-$2^d$) animals, thus demonstrating that Vβ6-bearing T cells in $CD4^{-/-}$ Mls-$1^a$ mice were deleted in the thymus. While $V\beta7^{hi}$ thymocytes were reduced in DBA/2 thymocytes, they were not reduced in $CD4^{-/-}$ Mls-$1^a$ mice. Control staining for $V\beta2^+$ T cells revealed that the mature population of $V\beta2^{hi}$ cells remained intact.

In vitro and in vivo studies have shown that $V\beta7^+$ T cells are the least reactive of all Mls-$1^a$-reactive Vβs described to date (14). In addition, the degree of reduction of $V\beta7^+$ T cells in $CD4^+$ Mls-$1^a$ mouse strains is not as drastic as that of $V\beta6^+$ T cells (9). The data demonstrating that CD4 expression is required for deletion of Vβ7-expressing T cells are consistent with these previous findings and supports a model in which Vβ7 has low affinity for Mls-$1^a$.

Reactivity against MHC class II–associated self-superantigens is more readily demonstrated with $CD4^+$ T cells expressing appropriate Vβs, however, both mature $CD4^+$ and $CD8^+$ T cells expressing these Vβs are deleted during thymic development (15). A contribution of CD4 in deletion mediated by MHC class II–associated self-superantigens was inferred from studies in which anti-CD4 treatment was able to prevent the deletion of $CD8^+$ T cells bearing self-superantigen-reactive Vβs (16, 17). Therefore, it was postulated that $CD8^+$ T cells expressing self-superantigen-reactive Vβs were targeted for deletion as a result of CD4 expression at the double-positive stage of thymocyte development. However, it has been subsequently demonstrated that $CD8^+$ T cells from $Mls^b$ mice are enriched for Vβ6, 8.1, and 9 expression after in vitro and in vivo Mls-$1^a$ stimulation (18–20), and that the in vitro anti-Mls-$1^a$ response of $CD8^+$ $V\beta6^+$ T cells is MHC class II dependent (21). Our results now demonstrate that CD4 expression is not absolutely required for Mls-$1^a$-mediated clonal deletion of $CD8^+$ $V\beta6^+$ T cells. However, the avidity required for clonal deletion of Mls-$1^a$-reactive TCRs may be attained through the interaction of CD8 with MHC class I in the thymus. To address this, experiments on clonal deletion in CD4/8-deficient mice are currently underway.

In summary, $CD8^+$ T cells expressing Vβ6, 8.1, and 9 TCRs are deleted from the periphery of $CD4^{-/-}$ Mls-$1^a$ mice. Analysis of Vβ expression on thymocytes has revealed that deletion of $V\beta6^+$ T cells can be detected in the thymus of $CD4^{-/-}$ Mls-$1^a$ mice. Thus, deletion of T cells with high affinity for Mls-$1^a$ determinants can occur in the absence of a signal through CD4. $V\beta7^+$ T cells were not deleted from the periphery or the thymus in $CD4^{-/-}$ Mls-$1^a$ mice. Therefore, there is a differential requirement for CD4 expression in clonal deletion such that CD4 expression is required for deletion when TCR affinity is reduced. There is evidence that T cell recognition of superantigens is different from recognition of nominal peptide antigen in the groove of MHC class II (22, 23). Whether or not the finding that CD4 expression is differentially required for Mls-$1^a$ self-superantigen-mediated deletion of T cells can be extended to deletion of T cells mediated by self-peptides presented in the groove of MHC class II requires further study.

We thank Dr. H. Acha-Orbea for providing the MMTV probe, Juliet Sheldon for flow cytometry advice, and Drs. K. Kishihara and M. Schilham for critical reading of the manuscript.

This work was supported by grants from the Medical Research Council and National Cancer Institute of Canada.

Address correspondence to Tak Mak, Department of Medical Biophysics and Immunology, University of Toronto, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada.

*Received for publication 27 May 1992 and in revised form 6 August 1992.*

## References


1. Blackman, M., J. Kappler, and P. Marrack. 1990. The role of the T cell receptor in positive and negative selection of developing T cells. *Science (Wash. DC).* 248:1335.
2. Acha-Orbea, H., A.N. Shakhov, L. Scarpellino, E. Kolb, V. Muller, A. Vessaz-Shaw, R. Fuchs, K. Blochlinger, P. Rollini, J. Billotte, M. Sarafidou, H.R. MacDonald, and H. Diggelmann. 1991. Clonal deletion of V$\beta$14-bearing T cells in mice transgenic for mammary tumour virus. *Nature (Lond.).* 350:207.
3. Beutner, U., W.N. Frankel, M.S. Cote, J.M. Coffin, and B.T. Huber. 1992. Mls-1 is encoded by the long terminal repeat open reading frame of the mouse mammary tumor provirus Mtv-7. *Proc. Natl. Acad. Sci. USA.* 89:5432.
4. Herman, A., J.W. Kappler, P. Marrack, and A.M. Pullen. 1991. Superantigens Mechanism of T-cell stimulation and role in immune responses. *Annu. Rev. Immunol.* 9:745.
5. Rahemtulla, A., W.P. Fung-Leung, M.W. Schilham, T.M. Kundig, S.R. Sambhara, A. Narendran, A. Arabian, D. Wakeham, C.J. Paige, R.M. Zinkernagel, R.F. Miller, and T.W. Mak. 1991. Normal development and function of CD8$^+$ cells but markedly decreased helper cell activity in mice lacking CD4. *Nature (Lond.).* 353:180.
6. Kozac, C., G. Peters, R. Pauley, V. Morris, R. Michalides, J. Dubdley, M. Green, M. Davisson, O. Prakash, A. Vaidya, J. Hilgers, A. Verstraeten, N. Hynes, H. Diggelmann, D. Peterson, J.C. Cohen, C. Dichkson, N. Sarkar, R. Nusse, H. Varmus, and R. Callahan. 1987. A standardized nomenclature for endogenous mouse mammary tumor viruses. *J. Virol.* 61:1651.
7. Groner, B., E. Buetti, H. Diggelmann, and N.E. Hynes. 1980. Characterization of endogenous and exogenous mouse mammary tumor virus proviral DNA with site-specific molecular clones. *J. Virol.* 36:734.
8. Payne, H., B.T. Huber, N.A. Cannon, R. Schneider, M.W. Schilham, H. Acha-Orbea, H.R. MacDonald, and H. Hengartner. 1988. Two monoclonal rat antibodies with specificity for the $\beta$-chain variable region V$\beta$6 of the murine T cell receptor. *Proc. Natl. Acad. Sci. USA.* 85:7695.
9. Okada, C.Y., B. Holzmann, C. Guidos, E. Palmer, and I.L. Weissman. 1990. Characterization of a rat monoclonal antibody specific for a determinant encoded by the V$\beta$7 gene segment. *J. Immunol.* 144:3473.
10. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. Kappler, and P. Marrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells. VI. An antibody to a receptor allotype. *J. Exp. Med.* 160:452.
11. Gregoire, C., N. Rebai, F. Schweisguth, A. Necker, G. Mazza, N. Auphan, A. Millward, A.-M. Schmitt-Verhulst, and B. Malissen. 1991. Engineered secreted T-cell receptor $\alpha\beta$ heterodimers. *Proc. Natl. Acad. Sci. USA.* 88:9077.
12. Staerz, U.D., H. Rammensee, J. Bendetto, and M. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
13. Utsunomiya, Y., H. Kosaka, and O. Kanagawa. 1991. Differential reactivity of V$\beta$9 T cells to minor lymphocyte stimulating antigen *in vitro* and *in vivo*. *Eur. J. Immunol.* 21:1007.
14. Waanders, G.A., and H.R. MacDonald. 1992. Hierarchy of responsiveness *in vivo* and *in vitro* among T cells expressing distinct Mls-1$^a$-reactive V$\beta$ domains. *Eur. J. Immunol.* 22:291.
15. MacDonald, H.R., A.L. Glasebrook, R. Schneider, R.K. Lees, H. Pircher, T. Pedrazzini, O. Kanagawa, J.-F. Nicolas, R.C. Howe, R.M. Zinkernagel, and H. Hengartner. 1989. T-cell reactivity and tolerance to Mls$^a$-encoded antigens. *Immunol. Rev.* 107:89.
16. Fowlkes, B.J., R.H. Schwartz, and D.M. Pardoll. 1988. Deletion of self reactive thymocytes occurs at a CD4$^+$8$^+$ precursor stage. *Nature (Lond.).* 334:620.
17. MacDonald, H.R., H. Hengartner, and T. Pedrazzini. 1988. Intrathymic deletion of self-reactive cells prevented by neonatal anti-CD4 antibody treatment. *Nature (Lond.).* 335:174.
18. Larsson-Sciard, E.L., A.L. Spetz-Hatgberg, A. Casrouge, and P. Kourilsky. 1990. Analysis of T cell receptor V$\beta$ gene usage in primary mixed lymphocyte reactions: evidence for directive usage by different antigen-presenting cells and Mls-like determinants on T cell blasts. *Eur. J. Immunol.* 20:1223.
19. MacDonald, H.R., R.K. Lees, and Y. Chvatchko. 1990. CD8$^+$ T cells respond clonally to Mls-1$^a$-encoded determinants. *J. Exp. Med.* 171:1381.
20. Webb, S.R., and J. Sprent. 1990. Response of mature unprimed CD8$^+$ T cells to Mls$^a$ determinants. *J. Exp. Med.* 171:953.
21. Chvatchko, Y., and H.R. MacDonald. 1991. CD8$^+$ T cell response to Mls-1$^a$ determinants involves major histocompatibility complex class II molecules. *J. Exp. Med.* 173:779.
22. Dellabona, P., J. Peccoud, C. Benoist, and D. Mathis. 1990. Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell.* 62:1115.
23. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor $\beta$ chain that interacts with the self-superantigen Mls$^a$. *Cell.* 61:1365.