Brief Definitive Report

CLONAL DELETION OF SELF-REACTIVE T CELLS IN IRRADIATION BONE MARROW CHIMERAS AND NEONATALLY TOLERANT MICE

Evidence for Intercellular Transfer of Mls^a

By DANIEL E. SPEISER,* RETO SCHNEIDER,* HANS HENGARTNER,* H. ROBSON MacDONALD,‡ AND ROLF M. ZINKERNAGEL*

*From the *Institute of Pathology, University Hospital, CH-8091 Zürich, Switzerland; and the ‡Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland*

The T cell repertoire is shaped by products of the MHC and by other self antigens. Presumed positive selection favors TCRs that are restricted to self MHC products (1–4); during a subsequent negative selection process T cells reactive to self antigens such as Mls^a (recently also designated Mls-1^a) are eliminated before they enter the thymic medulla (5, 6). Several experimental models suggest that induction of tolerance is MHC restricted (7, 8). In contrast, analyses of T cell reactivity against Mls^a suggest that Mls^a recognition is less closely correlated with class II MHC allele-specific restriction, although it clearly depends upon the presence of I-E products (9, 10). Because Mls^a is recognized together with I-E molecules of different MHC haplotypes and since the reacting T cells expressing Vβ6 (5) or Vβ8.1 (6) are present in most Mls^a-negative inbred mouse strains studied, it is expected that tolerance to Mls^a is not MHC restricted.

In this report, we used the mAb 44-22-1 that is specific for Vβ6 (11) and detects a T cell subset reactive to Mls^a (5) to analyze T cell recognition and mechanisms of induction and maintainance of tolerance in chimeras and neonatally tolerant mice exhibiting Mls^a and the permissive I-E on distinct cell populations. The data suggest that Mls^a may be transferred between cell compartments and that induction of tolerance to Mls^a requires presence of I-E but is I-E allele independent.

Materials and Methods

Animals. Inbred BALB/c (H-2^d), B10.D2 (H-2^d), DBA/2 (H-2^d), and CBA/J (H-2^k) mice were purchased from the Institute für Zuchthygiene, University of Zürich, Switzerland. DBA/1 (H-2^q) and B10.G (H-2^q) mice were obtained from Olac, Bicester, Oxon, U.K.

Chimeras. Bone marrow recipients were lethally irradiated (950 rad, 117 rad/min, ^{137}Cs source) and reconstituted 1 d later with 5–20 × 10^6 T cell-depleted bone marrow or fetal liver cells (1). The transplanted mice had a survival rate of 80–95%. Chimerism was monitored by FACS analysis with anti-H-2 class I mAbs.

Cytofluorographic Analysis. Aliquots of 10^6 lymphnode cells were stained at 4°C with rat

This work was supported in part by the Swiss National Science Foundation and the Radium Stiftung Zürich. Address correspondence to Rolf M. Zinkernagel, Institut für Pathologie, Abteilung für Experimentelle Pathologie, Sternwartstrasse 2, CH-8091 Zürich, Switzerland.

mAbs 44-22-1 (anti-Vβ6) (11) or KJ16-133 (anti-Vβ8.1/Vβ8.2) (6) followed by fluorescent goat anti-rat second reagent. Cortisone-resistant thymocytes (CRT; obtained 48 h after injection of 4 mg i.p. of hydrocortisone) were found to express comparable percentages of Vβ6 and Vβ8. For H-2 typing, nylon wool-purified spleen cells were incubated at 20°C with mAbs 100-5.28 (anti-K^kD^k) and 34-1-2 (anti-K^dD^d) (12). Samples were analyzed on an EPICS Profile flow cytometer (Coulter Electronics, Inc., Hialeah, FL).

^{51}Cr-release Assay. Vaccinia virus (Lancy strain; Schweizerisches Serum und Impfinstitut, Bern, Switzerland) was injected intravenously in a dose of 3×10^6 PFU. Infected mice were killed 6 d later and single spleen cell suspensions were tested for CTL activity on virus infected and uninfected ^{51}Cr-labeled target cells as described in detail elsewhere (1, 13).

Results and Discussion

Characterization and Examination of Vβ6 Usage in Bone Marrow Chimeras. In BALB/c →DBA/2 allogeneic chimeras where Mls^b ($H\text{-}2^d$) stem cells were used to reconstitute Mls^a ($H\text{-}2^d$) mice only low levels of $V\beta6^+$ T cells were found (Table I). Therefore host-derived radioresistant cells were capable of inducing tolerance to Mls^a. The converse combination, i.e., DBA/2 (Mls^a)→BALB/c (Mls^b), showed that Mls^a expression by lymphohemopoietic cells alone was also sufficient to induce tolerance. The same conclusions may be derived from observations in F1→parent chimeras (Table II). Thus, irradiated mice retain their ability to express Mls^a and tolerogenic Mls^a can be provided by chimeric donor- and host-type cells.

The lower percentages of $V\beta6^+$ or $V\beta8^+$ cells in bone marrow chimeras compared

TABLE I

Expression of Vβ6 and Vβ8 in Mls^b→Mls^a, Mls^a→Mls^b and Syngeneic Irradiation Bone Marrow Chimeras

Bone marrow donor	Bone marrow recipient		Donor H-2	Donor Mls	Recipient H-2	Recipient Mls	Percent lymph node cells expressing Vβ6 (Ab 44-22-1)	Percent lymph node cells expressing Vβ8 (Ab KJ16)
BALB/c →	DBA/2	(4)	d	b	d	a	0.8 ± 0.4	10.4 ± 0.4
DBA/2 →	BALB/c	(4)	d	a	d	b	0.8 ± 0.1	7.4 ± 0.3
BALB/c →	BALB/c	(4)	d	b	d	b	4.3 ± 0.5	7.7 ± 0.5
DBA/2 →	DBA/2	(4)	d	a	d	a	0.8 ± 0.4	8.1 ± 0.9
B10.G →	B10.G	(2)	q	b	q	b	2.4 ± 0.1	5.3 ± 0.6
DBA/1 →	DBA/1	(2)	q	a	q	a	2.5 ± 0.1	5.0 ± 0.0

Controls	IA	IE	H-2	Mls	Percent lymph node cells expressing Vβ6 (Ab 44-22-1)	Percent lymph node cells expressing Vβ8 (Ab KJ16)
B10.G	+	-	q	b	3.5	9.1
DBA/1	+	-	q	a	3.0	9.2
BALB/c	+	+	d	b	7.0	13.2
DBA/2	+	+	d	a	0.6	7.9
CBA/J	+	+	k	d	0.4	10.9

Chimeras were prepared as described in Materials and Methods and killed between 6 and 12 wk after transplantation. The numbers of transplanted mice analyzed are indicated in parentheses. Lymph nodes were used to prepare samples for FACS-analyses. The mean percentages and SEM of positive cells are given following subtraction of background values staining with the fluorescent anti-Ig conjugate alone. Total lymph node cell preparations contained between 50 and 65% Thy-1^+ cells. Expression of Lyt-1.1 was analyzed to trace DBA/2-derived cells: BALB/c → DBA/2 chimeras had 0.0% Lyt-1.1^+ lymph node cells (Lyt-1^+: 93.3 ± 1.5%). DBA/2 → BALB/c chimeras had 91.3 ± 1.2% Lyt-1.1^+ lymph node cells (Lyt-1^+: 87.3 ± 2.9%). H-2 and Mls typing as well as expression of I-A/I-E molecules were taken from the literature (17, 18).

TABLE II

Vβ6⁺ T Cells in F1 → Parent Irradiation Bone Marrow Chimeras

No.	Bone marrow donor	Bone marrow recipient	Donor H-2	Donor Mls	Recipient H-2	Recipient Mls	Percent lymph node cells expressing Vβ6 (44-22-1)	Percent lymph node cells expressing Vβ8 (KJ16)
1	(BALB/c × B10.G)F1→DBA/1	(3)	d×q	b×b	q	a	0.8 ± 0.0	5.2 ± 1.0
2	(BALB/c × B10.G)F1→DBA/2	(2)	d×q	b×b	d	a	0.9 ± 0.0	5.3 ± 0.0
3	(BALB/c × B10.G)F1→B10.G	(3)	d×q	b×b	q	b	3.5 ± 0.1	8.4 ± 1.0
4	(BALB/c × B10.G)F1→BALB/c	(2)	d×q	b×b	d	b	3.5 ± 0.3	5.0 ± 0.2
5	(DBA/2 × B10.G)F1 → DBA/1	(3)	d×q	a×b	q	a	0.7 ± 0.1	4.8 ± 0.2
6	(DBA/2 × B10.G)F1 → DBA/2	(4)	d×q	a×b	d	a	0.5 ± 0.2	6.1 ± 0.4
7	(DBA/1 × DBA/2)F1→B10.G	(3)	d×q	a×a	q	b	0.7 ± 0.0	6.8 ± 0.8
8	(DBA/1 × DBA/2)F1→B10.D2	(4)	d×q	a×a	d	b	0.6 ± 0.1	4.8 ± 0.5

For methods see legend to Table I. Percent spleen T cells expressing H-2^d/H-2^q analyzed by FACS were as follows: Group 1, 94/94; 2, 97/98; 3, 89/98; 4, 96/94; 5, 96/96; 6, 98/93; 7, 84/100; 8, 99/94. SEM was always <2.6%.

with untreated mice was partially because of the decreased proportion of T cells in lymph node preparations (~50 vs. 65% Thy-1⁺ cells); analysis was performed relatively soon after reconstitution of the chimeras. Since the lower percentages were found in syngeneically and semiallogeneically reconstituted animals, it probably reflects radiation damage and/or less efficient T cell maturation in chimeras.

Evidence for Intercellular Transfer of Mls^a in Irradiation Bone Marrow Chimeras. H-2^q mice possess Vβ6⁺ T cells, irrespective of Mls^a expression (Table I); the same is found in syngeneic control chimeras of this haplotype. Using H-2^d/I-E⁺ and H-2^q/I-E⁻ mice we prepared F1→parent chimeras in which Mls^a was only expressed by H-2^q-bearing (DBA/1) cells (Table II). Such cells lack I-E molecules and do not efficiently present Mls^a for either stimulatory response or for tolerance induction (9). These chimeras eliminated Vβ6⁺ T cells, demonstrating that for induction of tolerance Mls^a and I-E antigens may be provided by distinct cell subsets. Therefore, Mls^a had to be transferred to appropriate APCs.

I-E⁻ Mls^a Spleen Cells Induce Neonatal Tolerance in I-E⁺ Mls^b Recipients. The capacity of Mls^a spleen cells to induce neonatal tolerance was tested on (BALB/c × B10.G)F1 newborn mice; they were injected within 24 h of birth with 100 × 10^6 spleen cells of DBA/1 or DBA/2 mice. 2 wk later we analyzed Vβ6 and Vβ8 expression on CRT (Table III). As expected, neonatal injection of I-E⁺/Mls^a spleen cells (DBA/2) severely reduced Vβ6 expression in Mls^b recipients. Interestingly, I-E⁻/Mls^a spleen cells (DBA/1) reduced the expression of Vβ6 almost equally well, indicating that neonatal tolerance was induced to a considerable degree by transfer of Mls^a from I-E⁻ donor spleen cells to I-E⁺ APCs of the recipient.

The presented data confirm recent experiments by Pullen et al. (14), using a different mAb specific for Vβ3 that correlates with reactivity to Mls^c. Analysis of A (Mls^c, class II nonpermissive) + B (Mlsβ, class II permissive)→AxB (Mls^b) irradiation bone marrow chimeras revealed absence of Mls^c-specific Vβ3⁺ T cells in these chimeras. Our results are also compatible with in vitro studies published by DeKruyff et al. (15) showing that Mls^a-specific T cell clones proliferated when cocultured with DBA/1 stimulator B cells only if I-E⁺ splenic adherent cells were added.

TABLE III

Evidence for Intercellular Transfer of Mls^a during Neonatal Tolerance Induction In Vivo

Spleen cell donor	Recipient		Donor H-2	Donor Mls	Recipient H-2	Recipient Mls	Percent CRT expressing Vβ6 (Ab 44-22-1)	Percent CRT expressing Vβ8 (Ab KJ16)
DBA/1	(BALB/c × B10.G)F$_1$	(6)	q	a	d×q	b	2.3 ± 0.9	16.6 ± 1.8
DBA/2	(BALB/c × B10.G)F$_1$	(6)	d	a	d×q	b	2.1 ± 0.6	16.3 ± 1.7
BALB/c	(BALB/c × B10.G)F$_1$	(3)	d	b	d×q	b	10.2 ± 0.8	18.7 ± 2.1
None	(BALB/c × B10.G)F$_1$	(3)			d×q	b	11.1 ± 0.2	21.1 ± 0.3

F$_1$ mice were injected within 24 h of birth with 100 × 10^6 of the indicated donor spleen cells. After 2 wk, CRT containing 97.1 ± 1.1% CD3$^+$ cells were analyzed. Mean percentages and SD express the number of Vβ6$^+$ and Vβ8.1$^+$/Vβ8.2$^+$ T cells after subtraction of background values.

Dominant Restriction Specificity of T Cells Does Not Influence Mls^a-dependent TCR Vβ6 Usage in Chimeras. In the presence of Mls^a, H-2^d mice delete Vβ6$^+$ T cells, whereas in H-2^q mice, ~3–4% of mature T cells express Vβ6 (Table I). To evaluate the influence of the restriction specificity of T cells on tolerance induction, irradiation bone marrow chimeras of the following general type were made: (H-2^d × H-2^q)-F$_1$/Mls^{bxb} or Mls^{axb} or Mls^{axa} stem cells were used to reconstitute H-2^q/Mls^a or H-2^d/Mls^a or H-2^q/Mls^b or H-2^d/Mls^b irradiated recipients. The mice were typed for TCR Vβ6 expression (Table II) and for effector T cell restriction specificity (Table IV). After infection with vaccinia virus the bone marrow chimeras expressed anti-

TABLE IV

Antivaccinia Cytotoxic Response of Various Irradiation Bone Marrow Chimeras

6 d Vaccinia virus immune spleen cells from chimeras or control mice: Bone marrow donor		Bone marrow recipient	Killer/target ratio	Percent specific ^{51}Cr release of vaccinia virus-infected target cells: H-2^d(D2)	H-2^q(DBA/1)
(DBA/2 × B10.G)F$_1$	→	DBA/2	30	89	3
			10	79	3
			3	19	2
(DBA/1 × DBA/2)F$_1$	→	B10.G	30	3	92
			10	0	45
			3	0	7
(DBA/1 × DBA/2)F$_1$	→	B10.D2	30	96	1
			10	58	1
			3	29	2
(BALB/c × B10.G)F$_1$	→	DBA/2	30	70	7
			10	40	0
			3	11	2
		DBA/2	30	90	4
			10	53	1
			3	28	1
		B10.G	30	13	49
			10	4	41
			3	0	12

Test duration was 5 h; spontaneous release from infected D2, 15%; DBA/1, 19%.

viral T cell immunocompetence restricted predominantly to the H-2 haplotype of the thymus. In presence of Mls^a and I-E the chimeras had reduced levels of $V\beta6^+$ cells (Table II), no matter whether their T cells were restricted to $H\text{-}2^q$ or $H\text{-}2^d$. Therefore, selection of $V\beta6^+$ T cells apparently did not depend on the restriction specificity of effector T cells determined by the thymus. We obtained similar results with chimeras developing effector T cells restricted to $H\text{-}2^k$ (data not shown). Thus, tolerance induction to Mls^a is not I-E allele restricted, but generally I-E dependent.

The rules of Mls^a recognition and induction of tolerance shown here are compatible with the earlier findings that in $H\text{-}2^d$ and $H\text{-}2^q$ mice I-A is not involved, and the presence of I-E is necessary and sufficient for Mls^a recognition independent of the I-E allele (9, 16). The fact that Mls^a obviously does not exhibit the typical characteristics of T cell antigens, but still requires MHC molecules for presentation, may be called "pseudo-restriction" in contrast to the usually allele-specific restricted T cell recognition. This may be due to the low degree of polymorphism of I-E molecules (17).

In conclusion, our experiments are in agreement with the hypothesis of Mls^a being a peptide: as a whole or as fragments thereof it may be shed, reprocessed, and/or bound to I-E antigens. What remains unclear is why and how Mls^a peptides or Mls^a fragments stimulate such a high proportion of T cells.

Summary

Tolerance to Mls^a has been shown to be associated with clonal deletion of cells carrying TCR β chain variable regions $V\beta6$ or $V\beta8.1$ in mice possessing I-E antigens. To evaluate the rules of tolerance induction to Mls^a we prepared irradiation bone marrow chimeras expressing Mls^a or Mls^b and I-E by different cell types. Deletion of $V\beta6^+$, Mls^a-reactive T cells required the presence of Mls^a and I-E products either on bone marrow–derived cells or on irradiated recipient cells. Tolerance was induced when Mls^a and I-E were expressed by distinct cells of the chimera. Also neonatally tolerized mice exhibited depletion of $V\beta6^+$ cells after injection of $I\text{-}E^-$ Mls^a spleen cells (DBA/1) into newborn $I\text{-}E^+$ Mls^b mice $(BALB/c \times B10.G)F_1$. These results suggest that the product of the Mls^a locus is soluble and/or may be transferred from cell to cell and bound to I-E antigens.

The chimera experiments also showed that tolerance to Mls^a is H-2 allele independent, i.e., is apparently unrestricted. Differentiation of chimeric $(H\text{-}2^d/Mls^a \times H\text{-}2^q/Mls^b)F_1$ stem cells in either an $H\text{-}2^d$ or an $H\text{-}2^q$ thymus revealed that tolerance assessed by absence of $V\beta6^+$ T cells is not dependent on the thymically determined restriction specificity of T cells.

We thank Rosemary K. Lees, Andrée Porret, Alana Althage, and Suzanne Cooper for their excellent technical assistance.

Received for publication 4 January 1989 and in revised form 30 May 1989.

References

1. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882.

2. Kisielow, P., H. S. Teh, H. Blüthmann, and H. von Boehmer. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature (Lond.).* 335:730.
3. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature (Lond.).* 336:73.
4. MacDonald, H. R., R. K. Lees, R. Schneider, R. M. Zinkernagel, and H. Hengartner. 1988. Positive selection of $CD4^+$ thymocytes controlled by MHC class II gene products. *Nature (Lond.).* 336:471.
5. MacDonald, H. R., R. Schneider, R. K. Lees, R. C. Howe, H. Acha-Orbea, H, Festenstein, R. M. Zinkernagel, and H. Hengartner. 1988. T cell receptor $V\beta$ usage predicts reactivity and tolerance to Mls^a-encoded antigens. *Nature (Lond.).* 332:40.
6. Kappler, J. W., U. D. Staerz, J. White, and P. Marrack. 1988. Self tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (Lond.).* 332:35.
7. Groves, E. S., and A. Singer. 1983. Role of the H-2 complex in the induction of T cell tolerance to self minor histocompatibility antigens. *J. Exp. Med.* 158:1483.
8. Rammensee, H. G., and M. J. Bevan. 1984. Evidence from in vitro studies that tolerance to self antigens is MHC-restricted. *Nature (Lond.).* 308:741.
9. MacDonald, H. R., A. L. Glasebrook, R. Schneider, R. K. Lees, H. P. 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.
10. Janeway, C. A., Jr., and M. E. Katz. 1985. The immunobiology of the T cell response to Mls-locus-disparate stimulator cells. I. Unidirectionality, new strain combinations, and the role of Ia antigens. *J. Immunol.* 134:2057.
11. Payne, J., 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 $V\beta6$ region of the murine T cell receptor. *Proc. Natl. Acad. Sci. USA.* 85:7695.
12. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.
13. Cerottini, J. C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection and tumor immunity. *Adv. Immunol.* 18:67.
14. Pullen, A. M., P. Marrack, and J. W. Kappler. 1988. The T cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. *Nature (Lond.).* 335:796.
15. DeKruyff, R. H., S.-T. Ju, J. Laning, H. Cantor, and M. E. Dorf. 1986. Activation requirements of cloned inducer T cell. III. Need for two stimulator cells in the response of a cloned line to Mls determinants. *J. Immunol.* 137:1109.
16. Abe, R., and R. J. Hodes. 1988. The Mls system: non-MHC genes that encode strong T-cell stimulatory determinants. *Immunol. Today.* 9:230.
17. Bell, J. I., D. W. Jr. Denny, and H. O. McDevitt. 1985. Structure and polymorphism of murine and human class II major histocompatibility antigens. *Immunol. Rev.* 84:51.
18. Festenstein, H. 1976. The Mls system. *Transplant. Proc.* 8:339.