Major Histocompatibility Complex-specific Recognition of Mls-1 Is Mediated by Multiple Elements of the T Cell Receptor

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Summary

We have recently shown that recognition of the mouse mammary tumor virus 9-associated superantigen (vSAG-9) by murine Vβ17+ T cells is strongly influenced by the major histocompatibility complex (MHC) class II haplotype of the presenting cells, resulting in a form of MHC-restricted recognition. This finding was unexpected, because T cell recognition of another well-characterized retroviral superantigen, minor lymphocyte-stimulating antigen 1 (Mls-1), had been shown to be independent of the MHC haplotype of the presenting cell. To determine whether recognition of vSAG-9 and Mls-1 is fundamentally different, we undertook an extensive analysis of MHC haplotype influences on vSAG-9 and Mls-1 recognition by panels of T cell hybridomas. Our results show that, although most hybridomas recognized Mls-1 regardless of the MHC haplotype of the presenting cells, as previously described by others, some hybridomas exhibited unique patterns of MHC fine specificity. Thus, T cell recognition of vSAG-9 and Mls-1 is not fundamentally different, but the apparent differences can be explained in terms of frequency. The MHC fine specificity of individual Mls-1-reactive hybridomas was influenced by both Vβ and non-Vβ T cell receptor (TCR) elements. First, the influence of the Vβ element was apparent from the observation that Vβ8.2+ hybridomas were significantly more MHC specific in their recognition of Mls-1 than Vβ8.1 hybridomas. Second, a role for the TCR α chain was implicated from the distinct patterns of fine specificity of Mls-1 reactivity among a panel of transgenic hybridomas that expressed an identical β chain (Vβ8.1Dβ2Jβ2.3Cβ2). Sequence analysis revealed that junctional residues of the TCR α chain and/or Vα/Jα combinations influenced the MHC haplotype fine specificity for Mls-1. Third, DβJβ influences were implicated, in that the transgenic hybridomas expressed distinctive patterns of Mls-1 fine specificity not represented among Vβ8.1+ nontransgenic hybridomas. The findings that T cell recognition of endogenous superantigen is MHC specific, and that this specificity correlates with non-Vβ elements of the TCR, support the hypothesis that there is a direct interaction between the TCR and either polymorphic residues of the MHC class II molecule or haplotype-specific dominant peptides presented by class II.

Bacterial and retroviral superantigens are characterized by their ability to stimulate T cells based predominantly on the expression of specific TCR Vβ elements (1, 2), although non-Vβ elements of the receptor have recently been shown to have an influence on reactivity (3–6). MHC class II molecules are necessary for presentation of superantigens to T cells, but these responses are not classically MHC restricted, in that individual T cells are able to recognize a single superantigen presented in the context of multiple class II molecules. However, it has been shown that class II isotypes and alleles vary in their effectiveness as presenting molecules. For example, studies with murine clones and hybridomas specific for the mouse mammary tumor virus (Mtv)1-associated superantigen, Mls-1, have shown that I-E molecules tend to be stronger presenters than I-A molecules and that different MHC haplotypes can be ordered into a hierarchy where H-2k/H-2d are the strongest, and H-2b/H-2q are the weakest, presenters of Mls-1 (7–10). Based on these findings, it has been proposed that the class II molecule acts as a generic support for superantigen presentation at the cell surface and that the hierarchy of presentation reflects the relative binding affinity

1 Abbreviations used in this paper: Mtv, mouse mammary tumor virus; vSAG-9, Mtv-9-associated superantigen.
of individual superantigens to different MHC class II molecules (11, 12).

In contrast with this model, our recent studies with the Mtv-9-associated superantigen (vSAG-9) revealed that individual Vβ17+ T cell hybridomas were able to distinguish vSAG-9 presented by different MHC molecules, suggesting that under some circumstances, the class II molecule may play a more intimate role in the trimolecular interaction between superantigen, TCR, and MHC (13). The current studies were undertaken to determine why the recognition of vSAG-9 by T cell hybridomas should differ from the recognition of other retroviral superantigens, such as Mls-1. It is possible that this characteristic is unique to either the Vβ17 element of the TCR, or to the vSAG-9 moiety. Alternatively, individual Mls-1 reactive T cells may exhibit MHC haplotype specificity, but, because they are present at a lower frequency, have not been described. To distinguish these possibilities, we have analyzed panels of superantigen-reactive hybridomas for MHC haplotype specificity. We show here that the recognition of Mls-1 by some T cells is also critically dependent on the haplotype.


text continued...
A). Whereas some hybridomas recognized vSAG-9 in the reactive to Mls-1 presented in the context of all the MHC VB8.1 + and some VB8.2 + T cells have been shown to be Mls-1 reactive (9). To analyze the role of MHC molecules D1.LP). Mls-1 expression on the spleen cells was confirmed with a well-characterized hybridoma, 2HCa-2, that is strongly related with the other MHC haplotypes, H-2 k, H-2 a, and H-2 b (CBA/J, DBA/2, and BxH-7 (H-2 k, Mtv-9-) mice. Altogether, 96 Vβ5 + and 90 Vβ17 +/CD4 + hybridomas were identified that secreted IL-2 in response to immobilized anti-Vβ antibody. For simplicity, only the 40 Vβ5 + and 71 Vβ17+/CD4 + hybridomas that responded specifically to vSAG-9 presented by either H-2a and/or H-2b are shown. Each hybridoma was assayed two or three times to confirm the specificity pattern, and the results were always consistent with the initial analysis. The data presented are from the earliest assay of these hybridomas.

of both of these MHC haplotypes (13, and data not shown). Analysis of the Vβ5 + hybridomas revealed that only 40 of 96 (42%) responded to vSAG-9. Nonetheless, these hybridomas were strongly dependent on the MHC haplotype of the presenting cell in their recognition of vSAG-9 (Fig. 1 A). Whereas some hybridomas recognized vSAG-9 in the context of both H-2a and H-2b, others recognized vSAG-9 only in the context of one or the other of these haplotypes. These differences in fine specificity did not correlate with the expression of either Vβ5.1 or Vβ5.2 TCR elements (determined by MR9-4, anti-Vβ5.1/Vβ5.2 and MR9-8, anti-Vβ5.1 staining; data not shown) (26, 27). Thus, recognition of vSAG-9 by Vβ5 + T cells is highly dependent on the MHC haplotype of the presenting cell. The majority (71/90, 79%) of the Vβ17+/CD4 + hybridomas responded to vSAG-9, and many of these hybridomas recognized this superantigen in an MHC-specific manner, consistent with previous observations (Fig. 1 B).

Recognition of Mls-1 by Some T Cells Is MHC Specific. Most Vβ8.1 + and some Vβ8.2 + T cells have been shown to be Mls-1 reactive (9). To analyze the role of MHC molecules in this interaction, we generated a panel of Vβ8.1 + and Vβ8.2 + hybridomas from C57Bl/10 mice (H-2b, Mls-1 -). Altogether, we characterized 17 Vβ8.1+/CD4 + and 31 Vβ8.2+/CD4 + hybridomas for their reactivity to the Mls-1 antigen presented on spleen cells expressing three different MHC haplotypes, H-2a, H-2b, and H-2b (CBA/J, DBA/2, BALB/c, C57Bl/10). Mls-1 expression on the spleen cells was confirmed with a well-characterized hybridoma, 2HCa-2, that is strongly reactive to Mls-1 presented in the context of all the MHC haplotypes tested (6). Mls-1 specificity among the hybridomas was confirmed using MHC-matched spleen cells that do not express Mls-1 (CBA/Ca, B10.D2, C3H, B10.BR, BALB/c, C57Bl/10). All 17 of the Vβ8.1 + hybridomas recognized the Mls-1 superantigen. A comparison of reactivity of individual hybridomas to Mls-1 presented by H-2b vs. H-2a (Fig. 2 A) and H-2b vs. H-2b (Fig. 2 B) revealed no evidence of MHC-specific recognition of Mls-1, contrasting strongly with the MHC-dependent reactivity of the Vβ17 + and Vβ5 + hybridomas to vSAG-9. In general, each hybridoma responded more strongly to Mls-1 presented by H-2a and H-2b than to Mls-1 presented by H-2b, consistent with the findings of others that there is a hierarchy of reactivity to Mls-1 presented by different MHC alleles. The fact that there is a linear relationship between different MHC haplotypes in terms of their ability to present Mls-1 supports the concept that this hierarchy is controlled by the affinity of the Mls-1 molecule for different MHC class II alleles and not by individual TCRs (7–10).

In contrast to the Vβ8.1 + hybridomas, Vβ8.2 + hybridomas varied greatly in their recognition of Mls-1 presented by different MHC class II alleles (Fig. 2, C and D). Although only 12 of the 31 Vβ8.2 + hybridomas (39%) were Mls-1 reactive, several (7/12) of these hybridomas recognized Mls-1 presented exclusively by either H-2b or H-2d. Relatively few
(3/12) of the Vβ8.2 hybridomas recognized Mls-1 presented by H-2d. This MHC fine specificity could not be attributed simply to differences in the density of class II/Mls-1 molecules on the presenting cell since all of the presenting cells were strong stimulators of at least some of the hybridomas. Nor could the fine specificity of the hybridomas be attributed to differences in the density of TCR, CD4, or other adhesion molecules, since individual hybridomas had distinctive specificities inconsistent with variations in nonspecific adhesion molecules. Similarly, the fine specificity could not be attributed to the recognition of other superantigens, such as Mls-2/3 or vSAG-9, since the hybridomas did not respond to Mls-1 negative, MHC-matched C3H/HeJ (Mls-2/3+), B10.BR (vSAG-9+), or BALB/c (Mls-3+, vSAG-9+) spleen cells (data not shown). Taken together, these data establish that T cell recognition of the Mls-1 superantigen by some T cells is highly MHC specific. In addition, the differences in Mls-1 recognition by Vβ8.1+ vs. Vβ8.2+ T cells illustrates that the Vβ element of the TCR directly influences MHC-specific recognition of superantigen.

Interestingly, the frequency of Vβ8.2+ hybridomas that responded to Mls-1 in these studies (39%) was significantly higher than that reported in other studies (9, 35). This discrepancy may, in part, be explained by our screening procedures, which identify hybridomas specific for Mls-1 presented on MHC haplotypes other than H-2d. However, an additional factor is likely to be the LPS and IL-4 treatment of spleen cells, which greatly enhances Mls-1 expression (32, 33).

**MHC-specific Recognition of Mls-1 by Vβ8.1 Hybridomas from a Transgenic Mouse.** There are two possible explanations for the observation that Vβ8.2+ but not Vβ8.1+ T cell hybridomas recognize Mls-1 in an MHC-specific manner. First, it is possible that there is a fundamental difference in the recognition of Mls-1 by these two receptor elements. Alternatively, it is possible that there is a difference in the relative frequencies of MHC-specific, Mls-1-reactive cells. In the latter case, our failure to detect MHC-specific recognition of Mls-1 by Vβ8.1+ hybridomas may reflect a low frequency, rather than absence, of such cells. To determine whether at least some Vβ8.1 T cells can distinguish Mls-1 presented on different MHC molecules, we generated a panel of T cell hybridomas from a transgenic mouse (H-2d, Mls-1+) that expresses an identical Vβ8.1/D/β2/Jβ2.3/Cβ2 TCR β chain on essentially every T cell (6, 19). 75 Vβ8.1+ CD4+ hybridomas were analyzed for their ability to recognize Mls-1 presented in the context of three different MHC haplotypes, as described above. 60 of these hybridomas (80%) specifically recognized Mls-1. However, the pattern of fine specificity among the transgenic Vβ8.1+ hybridomas differed from that of the nontransgenic Vβ8.1+ hybridomas and was distributed into two major groups. Whereas some of the transgenic hybridomas recognized Mls-1 presented by all haplotypes tested (k, d, and b), other hybridomas recognized Mls-1 presented only in the context of k and b (Fig. 3, A and B). The distinction between these phenotypes is clearest in a comparison of recognition of Mls-1 presented by H-2d vs. H-2k (Fig. 3 B). The significant lack of H-2d specificity was especially surprising since H-2d is considered a strong presenter of Mls-1 to most Vβ8.1 T cells (9; see also Fig. 2, A and B). The finding that Vβ8.1 recognition of Mls-1 can also be strongly influenced by the MHC haplotype of the presenting cell indicates that there is no fundamental difference in recognition of Mls-1 by Vβ8.1+ and Vβ8.2+ T cells. In addition, the lack of MHC-specific recognition among nontransgenic Vβ8.1+ hybridomas suggests that these cells are represented at only a low frequency in normal mice but are amplified in the Vβ8.1 transgenic animals due to a skewing of the TCR repertoire.

**Figure 3.** IL-2 secretion (see legend to Fig. 1) by transgenic Vβ8.1+/CD4+ hybridomas (A and B) and transgenic Vβ8.1+/Vα2+/CD4+ hybridomas (C and D) in response to Mls-1 presented by CBA/J (Mls-1+, H-2d) vs. D1.LP (Mls-1+, H-2b) spleen cells (A and C) or DBA/2J (Mls-1+, H-2d) vs. D1.LP (Mls-1+, H-2b) spleen cells (B and D). Spleen cells were pretreated for 24 h with LPS and IL-4 to increase Mls-1 expression. Open symbols represent those hybridomas that expressed Vβ2 epitopes. All hybridomas were derived from Vβ8.1 transgenic mice in which the transgene has been repeatedly backcrossed onto CBA/CaJ (H-2d, Mls-1+) mice. Altogether, 75 Vβ8.1+/CD4+ hybridomas (derived from a single fusion of KJ16-activated T cells) and 45 Vβ8.1+/Vα2+/CD4+ hybridomas (derived from two separate fusions of B20.1-activated T cells) were identified that all secreted IL-2 in response to immobilized KJ16 (anti-Vβ8.1/Vβ8.2) antibody. For simplicity, only the 60 Vβ8.1+ hybridomas were assayed for their ability to recognize Mls-1 presented in the context of H-2d, H-2b, or H-2k are shown. Each hybridoma was assayed two or three times to confirm the specificity pattern, and the results were always consistent with the initial analysis. The data presented are from the earliest assay of these hybridomas.

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specific antibody. Hybridomas that expressed \( V\alpha 2^+ \) TCR are identified by open symbols in Fig. 3, A and B. The \( V\alpha 2^+ \) hybridomas were very unequally distributed between the two phenotypes, in that the majority (7/8) did not recognize Mls-1 in the context of H-2\( ^d \). These data establish that the \( V\alpha \) element of the TCR influences the MHC fine specificity of Mls-1 recognition.

To extend the correlation between fine specificity for Mls-1 and \( \alpha \) chain usage among the \( V\alpha 2^+ \) hybridomas, a larger panel of hybridomas was generated from transgenic T cells that had been activated with immobilized \( V\alpha 2 \)-specific mAb. Analysis of 29 \( V\alpha 2^-/CD4^+ \) hybridomas for their recognition of Mls-1 presented on different MHC haplotypes (Fig. 3, C and D) confirmed the finding that \( V\alpha 2^+ \) transgenic hybridomas had a distinct pattern of Mls-1 specificity. Again, the \( V\alpha 2^+ \) hybridomas were not equally distributed among the two phenotypes described for the unselected transgenic hybridomas, in that most of the hybridomas did not recognize Mls-1 presented in the context of H-2\( ^d \) (Fig. 3 D). Also, the \( V\alpha 2^+ \) hybridomas appeared to be more discriminatory in their recognition of Mls-1 presented by either \( b \) or \( k \) (Fig. 3 C). Inhibition studies with MHC class II-specific antibodies revealed that the two patterns of fine specificity did not correlate with the recognition of I-A vs. I-E, although interestingly, none of the hybridomas recognized Mls-1 exclusively in the context of I-A\( ^d \) (data not shown).

The Mls-1-reactive \( V\alpha 2^+ \) transgenic hybridomas could be classified into three distinct groups based on their specificity for Mls-1. Groups 1 and 2 were characterized by their preferential recognition of Mls-1 on either H-2\( ^k \) or H-2\( ^b \), but lack of response to Mls-1 presented by H-2\( ^d \). The third group was distinguished by the (nonexclusive) recognition of Mls-1 presented by H-2\( ^d \). To determine what component of the TCR \( \alpha \) chain controlled the three patterns of MHC specificity among the \( V\alpha 2 \) hybridomas, we identified the \( V\alpha \) family member, junctional, and \( J\alpha \) sequences in each hybridoma. As shown in Table 1, usage of particular \( V\alpha 2 \) family members did not correlate directly with fine specificity for Mls-1. For example, the \( V\alpha 2.3 \) and \( V\alpha 2.2 \) HCa8 elements were expressed frequently among hybridomas with distinctive patterns of Mls-1 recognition. Similarly, in general, there was no direct correlation between Mls-1 reactivity and \( J\alpha \) usage. However, there was a restricted use of \( J\alpha \) elements in group 1 hybridomas (\( k>b \)) in that of 17 of the hybridomas used \( J\alpha 17 \), \( J\alpha 24 \), and \( J\alpha 28 \) elements. These \( J\alpha \) elements were absent in Mls-1-reactive hybridomas with other patterns of fine specificity (groups 2 and 3). Interestingly, two hybridomas (4-77 and 4-59) that differed only in junctional residues expressed distinctive patterns of fine specificity for Mls-1. This is consistent with previous studies that have implicated \( \alpha \) chain junctional sequences in Mls-1 reactivity of transgenic hybridomas (6). Taken together, these data demonstrate that different elements of the \( \alpha \) chain contribute to the MHC fine specificity of Mls-1 recognition, although we have not identified a simple correlation between MHC specificity and usage of particular \( \alpha \) chain elements. These observations are consistent with the hypothesis that there is a direct interaction between the MHC molecule and TCR during superantigen engagement (11, 13–18).

**Discussion**

Our previous studies had shown that recognition of the Mtv-9-associated superantigen, vSAG-9, by \( V\beta 5^+ \) T cells was strongly influenced by the MHC haplotype of the presenting cell (13). However, this finding contrasted with the large body of work regarding T cell recognition of another retroviral superantigen, Mls-1. Although different MHC molecules clearly varied in their capacity to present Mls-1, individual hybridomas or clones were not reported to distinguish between Mls-1 moieties presented on different MHC molecules (7–10). To better understand the differences between T cell recognition of vSAG-9 and Mls-1, we have analyzed the specificities of several panels of hybridomas and shown that the Mls-1 superantigen can also be recognized in an MHC-specific fashion by some T cells. Thus, these studies demonstrate that there is no fundamental difference in the
Table 1. Sequence Analysis of Mls-1-reactive, Vα2⁺ Hybridomas Derived from a Vβ8.1 Transgenic Mouse

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* The Vα2⁺ hybridomas presented here are derived from three separate fusions, designated by the prefixes 4, 5, and 6 in the hybridoma name. All hybridomas were generated from Vβ8.1 transgenic CD4⁺ cells that were activated with either B20.1 (anti-Vα2, fusions 4 and 6; shown in Fig. 3, C and D) or KJ16 (anti-Vβ8.1/Vβ8.2, fusion 5; shown in Fig. 3, A and B). Hybridoma reactivity is presented as IL-2 secretion in response to Mls-1⁺ spleen cells of the indicated MHC haplotypes or immobilized KJ16 antibody. IL-2 assays and TCR α chain sequencing was performed as described in Materials and Methods. The groupings k>b, b>k, and d-reactive indicate the relative MHC specificity of the hybridomas for Mls-1.

† The sequences of Vα2. HCa8, Vα2.3, and Vα2.5 have been previously published (6, 36, 37). The sequences of 4HCa-19, 4HCa-75, and 4HCa-101 Vα2 elements will be published separately.

‡ Amino acid residues in parentheses were encoded by the Vα gene. [NI], not identified.
Figure 4. IL-2 secretion by Vβ5+ hybridomas derived from (A) B10.BR (H-2k, Mtv-9+) and (B) BxH-7 (H-2k, Mtv-9-) mice in response to the Mtv-9-associated superantigen, vSAG-9, presented by either (H-2k, Mtv-9+) and (B) BxH-7 (H-2k, Mtv-9-) mice in response to the analysis. The data presented are from the earliest of these hybridomas. The data for the BxH-7 hybridomas are from Fig. 1 and are shown for comparison. All of the hybridomas presented here secreted IL-2 in response to immobilized MR.9-4 (anti-V35) antibody even though many of them did not specifically respond to vSAG-9 presented on the transfectants. Each hybridoma was assayed two or three times to confirm the specificity pattern, and the results were always consistent with the initial analysis. The data presented are from the earliest of these hybridomas.

recognition of these two superantigens, but that the frequency of MHC-specific, Mls-1-reactive T cells is low.

We and others have previously shown influences of non-Vβ elements of the TCR on endogenous superantigen recognition (3–6). The data presented here extend these studies to show that these non-Vβ influences correlate with the MHC fine specificity of individual hybridomas. First, a direct role for the TCR α chain was apparent from the distinct patterns of Mls-1 fine specificity among Vβ8.1+ transgenic hybridomas, which all expressed an identical β chain. Sequence analysis of some of these hybridomas did not reveal a direct correlation between specificity and Vα family member usage or Jα usage, suggesting that junctional residues or combinations of these elements control this reactivity. Second, a role for non-Vβ elements of the β chain in Mls-1 fine specificity was apparent from the observation that the Vβ8.1+ transgenic hybridomas expressed distinct patterns of MHC specificity that were not found among nontransgenic Vβ8.1+ hybridomas. It is likely that restriction of the repertoire to a single transgenic β chain has amplified a minor population of T cells with distinctive specificities in the transgenic animals.

Although the molecular mechanism underlying MHC-specific recognition of retroviral superantigens remains to be determined, the involvement of non-Vβ elements suggests two possibilities. One possibility is that non-Vβ elements affect the binding of superantigen to the TCR, either directly by contacting superantigen or indirectly by inducing a conformational change in the superantigen binding site on Vβ. However, this mechanism is unlikely to explain differences in MHC specificity as reported here. An alternative possibility, and one that we and others have proposed previously, is that MHC-specific recognition of superantigen is mediated by a direct interaction between the TCR and the MHC class II molecule during the formation of a superantigen/TCR/MHC complex (11, 13–18). For example, interactions between polymorphic residues of the MHC molecule (and/or bound peptide) and the α chain of the TCR might act to either stabilize or disrupt formation of a superantigen/TCR/MHC complex. In the case of the β chain, non-Vβ components could affect fine specificity either by selecting a repertoire of α chains that confer distinctive Mls-1 specificities on the T cell, or by directly contacting the MHC molecule itself. We are further investigating these proposed interactions by mutagenesis experiments in which mutations in the TCR, MHC class II molecules, and Mls-1 will be analyzed for their influence on MHC-restricted recognition.

In the absence of structural information, it is difficult to predict how non-Vβ elements might interact with the MHC during superantigen recognition (39, 40). One possibility is that the interaction is the same as during recognition of conventional antigen/MHC. Alternatively, it is possible that the TCR/MHC interaction is unconventional comes from our study of Mls-1-reactive, Vβ8.2+ hybridomas. Since these hybridomas were derived from a C57Bl/10 mouse, the parental T cells should have been positively selected by, and presumably have a weak affinity for, H-2b molecules. If favorable TCR/MHC interactions are required for the recognition of Mls-1 by Vβ8.2+ T cells, then one might have expected to see significant numbers of hybridomas that preferentially recognized Mls-1 in the context of H-2b. However, this was not the case, suggesting that positive selection in the thymus had not directly influenced the fine specificity of the T cells to Mls-1, and supporting the idea that TCR/MHC interactions differ between superantigen and classical peptide recognition. An unconventional TCR/MHC interaction during superantigen engagement might explain reports suggesting that signaling events associated with TCR recognition of superantigen are different from those induced by recognition of conventional antigen/MHC (41–43).

An important question raised by these studies is why superantigen recognition of only some T cell hybridomas is MHC specific. We have previously proposed that this reflects differences in affinity between the superantigen and TCR (13). Thus, if the affinity between the TCR and the superantigen is weak, the stability of the MHC/TCR/superantigen complex is likely to be strongly influenced by stabilizing or disruptive interactions between the TCR and polymorphic residues of MHC (and/or bound peptide), resulting in a high frequency of MHC specificity. In contrast, if the TCR/superantigen interaction is strong, direct TCR/MHC interactions are likely to have less influence on the stability of the trimolecular complex, thus resulting in a lower frequency of MHC specificity. In this case, recognition of superantigen would reflect the hierarchy that is thought to be controlled by variation in the affinity of the superantigen for different MHC class II molecules (12). The finding that MHC-specific
recognition of Mls-1 was frequent among Vβ8.2+ and infrequent among Vβ8.1+ hybridomas is consistent with this hypothesis. Although it is not yet possible to directly measure the affinities of individual TCRs for Mls-1, several observations indirectly suggest that Vβ8.1+ T cells are generally of higher affinity than Vβ8.2+ T cells. First, a much higher frequency of Vβ8.1+ hybridomas respond to Mls-1 (in these experiments, 100% of Vβ8.1 hybridomas, compared with 39% of Vβ8.2+ hybridomas, recognized Mls-1 on the MHC haplotypes tested). Second, Vβ8.1+ T cells are effectively clonally eliminated from the periphery of Mls-1-expressing mice, whereas Vβ8.2+ T cells are relatively unaffected (9). Third, mutational analysis has suggested that glycosylation sites on the Vβ8.2 element partially obscure the Mls-1 binding site. Removal of these sites by mutation restores Mls-1 recognition in some, but not all, Vβ8.2+ TCRs (35, 44). Additional support for the hypothesis comes from the unexpected high frequency of MHC specificity of the Vβ8.1 transgenic hybridomas. For reasons that are as yet unclear, this particular β chain appears to have a relatively low affinity for Mls-1. For example, Mls-1 mediates only poor clonal deletion of transgenic T cells in these animals (6, 19) and in the studies presented here, only 80% of transgenic T cells were Mls-1 reactive. Thus, apparent differences in affinity of Vβ8.1, Vβ8.2, and transgenic Vβ8.1 hybridomas for Mls-1 correlate with the different degrees of MHC specificity in their Mls-1 recognition.

The finding that individual T cells have different fine specificities for retroviral superantigens has significance for repertoire selection in vivo. We speculated that some T cells predicted to be superantigen reactive on the basis of their Vβ usage would fail to be clonally eliminated during ontogeny because they were unable to recognize the superantigen presented in the context of self-MHC. Analysis of vSAG-9 reactivity among Vβ5+ hybridomas from B10.BR (Mtv-9+, H-23) mice revealed that while vSAG-9/H-23 reactivity had been purged (either by deletion or anergy), it was still possible to detect vSAG-9/H-23 reactivity. These data suggest that clonal deletion is incomplete, at least in part, because some T cells do not recognize the superantigen presented in the context of self-MHC molecules. This hypothesis is difficult to test directly due to the interference of strong alloreactive responses and the possibility that the activation of T cells by Mls-1 on syngeneic and allogeneic MHC may induce alternative activation pathways. For example, it has been reported that neonatal induction of tolerance to Mls-1 with allogeneic spleen cells does not result in the peripheral deletion of Mls-1-reactive T cells, contrasting with the strong clonal deletion of Mls-1-reactive T cells induced by syngeneic spleen cells (45, 46).

Taken together, the data presented in this paper show that T cell recognition of retroviral superantigens, such as vSAG-9 and Mls-1, can be strongly influenced by the MHC molecule, resulting in distinct patterns of fine specificity. Moreover, the clear involvement of both α and β chain elements of the TCR suggests that there is a direct interaction between the TCR and MHC molecule during superantigen engagement. These findings have great significance for our understanding of T cell reactivity and tolerance to retroviral superantigens.

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