Brief Definitive Report

CD8$^+$ T CELLS RESPOND CLONALLY TO Mls-1^a-ENCODED DETERMINANTS

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The gene product of the minor lymphocyte stimulatory locus Mls-1^a is responsible for the activation of a large proportion (up to 20%) of T lymphocytes in unprimed mice (1, 2). Recent studies indicate that at least three independent TCR Vβ domains (Vβ6, Vβ8.1, and Vβ9) confer preferential reactivity of T cells to Mls-1^a determinants, and that mature T cells bearing these Vβ are clonally deleted in Mls-1^a strains (3–5). Thus, this Vβ preference probably accounts for the high frequency of Mls-1^a-reactive T cells.

In analogy with responses of CD4$^+$ T cells to conventional peptide antigens, T cell reactivity to Mls-1^a requires the coexpression of MHC class II molecules (particularly I-E) on the stimulating cells and can be blocked by mAbs directed against MHC class II. Furthermore, essentially all published T cell clones and hybridomas exhibiting anti-Mls-1^a reactivity are CD4$^+$, and responsiveness of polyclonal T cell populations to Mls-1^a can be completely inhibited by anti-CD4 mAbs. These findings have been collectively interpreted as demonstrating that Mls-1^a reactivity is restricted to the CD4$^+$ T cell subset (6).

In this work, we have reinvestigated the response of CD8$^+$ T cells to Mls-1^a determinants. Taking advantage of the availability of Mls-1 congenic mouse strains (7), we find that CD8$^+$ cells using Vβ6$^+$ and Vβ8.1$^+$ TCRs are dramatically enriched after in vitro or (to a lesser extent) in vivo stimulation by Mls-1^a. The parallel selection of TCR Vβ domains in both CD4$^+$ and CD8$^+$ subsets by Mls-1^a is reminiscent of T cell responses to other MHC class II–dependent "superantigens" such as bacterial enterotoxins.

Materials and Methods

Mice. Congenic BALB/c (H-2^d, Mls-1^b) and BALB.D2.Mls^a (H-2^d, Mls-1^a) mice were maintained from breeding pairs kindly provided by Dr. H. Festenstein (7). DBA/2 (H-2^d, Mls-1^a), DBA/1 (H-2^q, Mls-1^a), and CBA/J (H-2^k, Mls-1^a) mice were obtained from Harlan-Olac U.K., Bicester, UK. F_1 hybrids between BALB/c and either CBA/Ca (H-2^k, Mls-1^b) or B10.G (H-2^q, Mls-1^b) mice were bred locally.

Mixed Leukocyte Cultures. Nylon wool–purified responder splenic T cells (1.5×10^6) were cultured with irradiated (1,000 rad) anti-Thy-1 plus complement–depleted splenic stimulator cells (4.5×10^6) in 2 ml DME supplemented with 5% FCS and 5×10^{-5} M 2-ME. In some experiments, responder populations were depleted of CD4$^+$ or CD8$^+$ cells by further treat-

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ment with rat IgM mAbs RL172.4 (anti-CD4) or 3.168.1 (anti-CD8) plus rabbit complement before culture. In the case of CD4 depletion, the resulting $CD8^+$ responder cells were further supplemented with supernatant of PMA-stimulated EL4-6.1 cells (corresponding to 30 U/ml IL-2).

Blast Purification. After 3 d in culture, responding T cell blasts were isolated on a Percoll density gradient (8) and resuspended (2×10^5 viable cells/ml) in fresh medium supplemented with human rIL-2 (60 ng/ml). After a further 2–3 d (during which time cell density increased 7–10-fold), cells were recovered and analyzed for $V\beta$ expression.

In Vivo Transfers. BALB/c mice were irradiated (850 rad from a ^{137}Cs source). After 20 h, mixtures of purified T cells and (unirradiated) B cells were injected intravenously into groups of two mice. Spleens were recovered and pooled 7–9 d later, and T cells were repurified on nylon wool columns before analysis of $V\beta$ expression. Survival was 100% in all experiments.

Flow Microfluorometry. All procedures and reagents have been described (3). Cells were initially stained with TCR $V\beta$-specific mAbs 44-22-1 (anti-$V\beta_6$), KJ16 (anti-$V\beta_{8.1/8.2}$), and F23.2 (anti-$V\beta_{8.2}$) followed by appropriate fluoresceinated anti-Ig. Additional staining was with phycoerythrin-conjugated GK-1.5 (anti-CD4) or biotinylated 53-6.7 (anti-CD8; revealed with avidin-PE).

Fluorescence histograms for $V\beta$ staining were gated independently on $CD4^+$ or $CD8^+$ cells (see Fig. 1 for example). The percentage of positive cells in each subset expressing each $V\beta$ was calculated directly from the gated histograms (except for $V\beta_{8.1}$, which was determined by subtraction of F23.2 staining from KJ16 staining).

Results and Discussion

To assess the possibility that $CD8^+$ T cells may respond to Mls-1^a-encoded determinants, we took advantage of the availability of the congenic mouse strains BALB/c and BALB.D2.Mls^a (7). Purified T lymphocytes from BALB/c (Mls-1^b) mice were stimulated with lightly irradiated Mls-1^a congenic B cells (T-depleted spleen), and the resulting T cell blasts were isolated and recultured in rIL-2. This expanded population was then double stained with mAbs directed against TCR $V\beta$ domains and either CD4 or CD8. As expected from previous studies (3, 4), the $CD4^+$ blast subset was highly enriched in cells expressing $V\beta_6$ and $V\beta_{8.1}$ after Mls-1^a stimulation (Fig. 1, Table I). Surprisingly, similar analysis of the $CD8^+$ subset revealed almost identical proportions of cells expressing each of these $V\beta$ elements (Fig. 1, Table I). In each case, $V\beta_6^+$ cells were enriched five- to sixfold and $V\beta_{8.1}$ cells two- to fourfold as compared with the control (unstimulated) subset. This pattern of $V\beta$ expression among $CD8^+$ cells was dependent upon Mls-1^a stimulation since control stimulation (with syngeneic Mls-1^b splenic B cells) resulted in proportions of $V\beta_6^+$ and $V\beta_{8.1}^+$ cells similar to those found in the freshly isolated population (Table I). Fur-

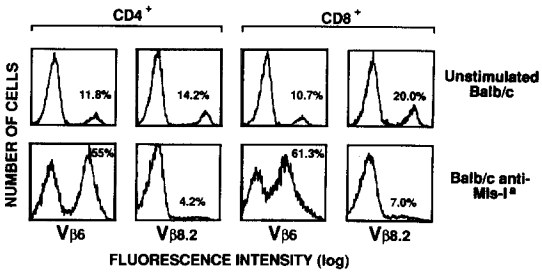

FIGURE 1. TCR $V\beta$ expression by $CD4^+$ and $CD8^+$ T cell subsets after Mls-1^a stimulation in vitro. BALB/c anti-Mls-1^a T cell blasts were isolated and double stained with anti-TCR $V\beta$ mAbs together with either anti-CD4 or anti-CD8 mAbs. Histograms shown are gated on either $CD4^+$ or $CD8^+$ cells.

TABLE I

Expression of TCR Vβ Domains by CD4⁺ and CD8⁺ T Cell Blasts Responding to Mls-1ᵃ Determinants In Vitro

Mixed leukocyte culture				Proportion of CD4⁺			Proportion of CD8⁺		
Responder	Stimulator	CD4⁺	CD8⁺	Vβ6	Vβ8.1	Vβ8.2	Vβ6	Vβ8.1	Vβ8.2
				%					
BALB/c	BALB/c	68	16	12.7	7.0	15.0	10.4	8.7	20.0
BALB/c	BALB.D2.Mls^a	75	15	55.5	27.3	4.2	64.4	19.5	7.0
BALB/c (CD8⁺)*	BALB.D2.Mls^a	<1	64	-	-	-	64.0	ND	ND
CBA/Ca × BALB/c	None	53	33	11.7	4.8	14.8	12.9	8.1	16.4
CBA/Ca × BALB/c	BALB.D2.Mls^a	76	15	69.7	22.6	2.2	62.7	15.9	2.6
CBA/Ca × BALB/c	CBA/J	76	16	67.8	26.2	3.1	56.3	28.4	2.4
CBA/Ca × BALB/c (CD4⁺)*	BALB.D2.Mls^a	98	<1	72.5	18.0	3.4	-	-	-
CBA/Ca × BALB/c (CD8⁺)*	BALB.D2.Mls^a	<1	80	-	-	-	57.3	14.7	6.2
CBA/Ca × BALB/c (CD8⁺)*	BALB/c	<1	49	-	-	-	9.0	12.6	19.0
B10.G × BALB/c	None	46	29	10.4	1.5	21.4	12.1	9.4	14.3
B10.G × BALB/c (CD4⁺)*	DBA/2	95	ND	66.1	31.1	3.5	-	-	-
B10.G × BALB/c (CD4⁺)*	DBA/1	76	ND	22.2	8.6	22.4	-	-	-
B10.G × BALB/c (CD8⁺)*	DBA/2	ND	90	-	-	-	66.9	12.4	2.6
B10.G × BALB/c (CD8⁺)*	DBA/1	ND	65	-	-	-	10.0	8.6	14.6

Nylon wool-purified splenic responder T cells (1.5×10^6) were mixed with irradiated (1,000 rad) T cell-depleted splenic stimulator cells (4.5×10^6). After 3 d, responding T blasts were isolated on a Percoll gradient and recultured for 2–3 d in rIL-2 (60 ng/ml). Recovered cells were double stained with mAbs directed against the indicated TCR Vβ domains and either CD4 or CD8 (see Fig. 1).

* Responding T cells were CD4 depleted (CD8⁺) or CD8 depleted (CD4⁺) before culture. For subsequent stimulation of CD8⁺ T cells, IL-2 (30 U/ml in the form of EL4 supernatant) was added from the outset.

thermore, enrichment of Mls-1^a-specific CD8⁺ Vβ6⁺ cells did not require the simultaneous activation of CD4⁺ cells as shown by CD4 depletion experiments (Table I).

The response of CD4⁺ T cell clones and hybridomas to Mls-1^a determinants depends upon coexpression of MHC class II molecules by the stimulating cells, with a marked preference for H-2^k and H-2^d haplotypes and essentially no response to H-2^q (2). As shown in Table I, CD8⁺ Vβ6⁺ cells from appropriate (Mls-1^b)F$_1$ mice responded preferentially to Mls-1^a in the context of H-2^d (BALB.D2.Mls^a or DBA/2) or H-2^k (CBA/J) stimulator cells, but no such selection was seen with H-2^q (DBA/1). Predictably, the response of CD4⁺ cells followed essentially the same pattern (Table I), although a small but significant (twofold) enrichment in Vβ6⁺ cells was also seen with DBA/1 stimulator cells. This latter result may reflect a weak stimulation of CD4⁺ cells by Mls-1^a in the context of H-2^q; alternatively, Mls-1^a may be reprocessed and presented by residual (I-E⁺)F$_1$ cells, as shown previously for a T cell clone (9). In either event, the fact that clonal responses of both CD4⁺ and CD8⁺ T cells to Mls-1^a were dependent upon the same MHC alleles raises the possibility that class II molecules are also involved in Mls-1^a-specific stimulation of the CD8⁺ subset. More direct experiments will be required to confirm this hypothesis.

Since reactivity of CD8⁺ T cells to Mls-1^a was somewhat unexpected, we also investigated this response in an adoptive transfer system in vivo. Preliminary experiments established that reproducible responses to Mls-1^a in irradiated hosts required coinjection of both Mls-1^b T cells and Mls-1^a B cells, presumably because B cells

responsible for Mls-1^a stimulation are highly radiosensitive (10). Using this system, we determined the Vβ composition of splenic T cell populations derived from irradiated BALB/c mice given varying doses of syngeneic (BALB/c) T cells and a constant number (3–4 × 10^7) of congenic BALB.Mls^a B cells. The result of this analysis for CD4$^+$ T cells (Table II) demonstrated a dramatic increase in Vβ6 and Vβ8.1 expression over a wide (~30-fold) range of donor T cell doses. Thus, Vβ6$^+$ cells accounted for 52% of the total CD4$^+$ population (similar to the in vitro data), whereas Vβ8.1$^+$ cells accounted for 10–12%. Results obtained in this system for CD8$^+$ T cells were considerably more variable (Table II); in fact, significant numbers of these cells were not obtained in all experiments, even at the highest donor T cell doses. Nevertheless, when CD8$^+$ cells were present, Vβ6$^+$ and Vβ8.1$^+$ cells were significantly enriched (about twofold). It should be noted that alterations in Vβ expression in both CD4$^+$ and CD8$^+$ subsets were again strictly dependent upon the presence of Mls-1^a B cells, since animals reconstituted with a mixture of syngeneic (BALB/c) T and B cells did not differ from unmanipulated controls (Table II).

The apparently reduced efficiency of Mls-1^a-specific stimulation of CD8$^+$ cells in the in vivo model system (as compared with mixed leukocyte cultures) requires further analysis. In this regard, in vitro stimulation of CD8$^+$ Mls-1^a-specific cells may be favored by the presence of IL-2, since we were unable to detect preferential expansion of Vβ6$^+$ or Vβ8.1$^+$ cells using CD4-depleted responder populations unless exogenous IL-2 was added (data not shown).

The data presented here do not support the generally accepted dogma that only CD4$^+$ T cells respond to Mls-1^a determinants (6), nor are they consistent with recent evidence that CD8 expression inhibits responses to MHC class II–restricted

TABLE II

Selective TCR Vβ Expression on CD4$^+$ and CD8$^+$ Cells Responding to Mls-1^a Determinants In Vivo

Cells transferred				Proportion of CD4$^+$			Proportion of CD8$^+$		
Responder	Stimulator	CD4$^+$	CD8$^+$	Vβ6	Vβ8.1	Vβ8.2	Vβ6	Vβ8.1	Vβ8.2
				%					
5 × 10^6 BALB/c	BALB/c	60	12	13.5	8.9	15.6	11.8	5.7	22.7
1.5 × 10^7 BALB/c	BALB.D2.Mls^a	69	14	52.3	10.2	7.8	25.3	11.0	19.6
				52.6	11.1	8.1	21.4	7.8	25.9
				52.5	10.8	7.1	-*	-	-
5 × 10^6 BALB/c	BALB.D2.Mls^a	51	8	53.3	11.3	8.4	22.9	10.0	19.3
				52.5	13.6	6.2	-	-	-
1.5 × 10^6 BALB/c	BALB.D2.Mls^a	54	4	52.8	10.8	7.6	22.8	13.9	17.3
5 × 10^5 BALB/c	BALB.D2.Mls^a	38	<1	48.3	10.7	8.1	-	-	-
5 × 10^4 BALB/c	BALB.D2.Mls^a	40	<1	19.6	3.7	14.5	-	-	-
None	BALB.D2.Mls^a	24	<1	6.9	3.9	16.1	-	-	-

Irradiated BALB/c mice (two mice per group) were reconstituted 20 h later with varying numbers of responder BALB/c splenic T cells and a constant number (3.5 × 10^7) of BALB.D2.Mls^a (or control BALB/c) T cell–depleted splenic stimulator cells. After 7–9 d, CD4$^+$ and CD8$^+$ splenic T cells from reconstituted mice were analyzed for Vβ expression as in Fig. 1. Control values for normal BALB/c mice are given in Table I (first line).

* Insufficient CD8$^+$ cells for analysis (<1%).

antigens (as well as Mls-1^a) in transfected T cell hybrids (11). Nevertheless, our results are compatible with an isolated report in which proliferation of an MHC class I-restricted CD8$^+$ clone specific for influenza virus was found to correlate with Mls-1^a expression in the absence of nominal antigen (12). Moreover, increased Vβ_6 and V$\beta_{8.1}$ usage has recently been observed among CD8$^+$ T cells stimulated with MHC-incompatible T cell blasts derived from Mls-1^a strains (13). Although the latter system is more complex than ours (involving both MHC and minor antigen differences in addition to Mls-1^a), both studies point to a generalized Vβ-specific clonal response of CD8$^+$ T cells to Mls-1^a determinants.

Finally, in a broader context, the parallel Vβ usage among CD4$^+$ and CD8$^+$ T cells responding to Mls-1^a may reflect a more general property of the class of mitogenic substances currently referred to as superantigens (14). In this regard, the Staphylococcal enterotoxins (the only superantigens yet defined in molecular terms) also elicit MHC class II-dependent responses from both CD4$^+$ and CD8$^+$ T cells (15), and recent data indicate that Vβ usage is similar in both subsets (16). Furthermore, I-E-dependent responses of V$\beta_{11}{}^+$ and V$\beta_{17a}{}^+$ T cells (which involve undefined ligands functionally analogous to superantigens) can be mediated by CD8$^+$ cells in certain instances (17, 18). Since TCRs on all CD8$^+$ mature T cells are presumably selected for MHC class I ($\pm$ peptide?) recognition during thymic development (19), the preferential use of certain Vβ domains by these cells in MHC class II-dependent responses to superantigens is most easily reconciled with models involving direct TCR-superantigen interaction. One possibility might be that superantigens first bind to MHC class II molecules in order to present determinant(s) that can interact effectively with the appropriate TCR Vβ domains.

Summary

T cell responses to the product of the minor lymphocyte stimulatory locus Mls-1^a involve the selective use of TCR Vβ domains (especially Vβ_6 and V$\beta_{8.1}$) and are generally considered to be restricted to the CD4$^+$ mature subset. We show here that CD8$^+$ (presumably MHC class I-restricted) T cells bearing Vβ_6 or V$\beta_{8.1}$ also respond preferentially to Mls-1^a determinants either in vitro (in mixed leukocyte cultures) or in vivo (in an adoptive transfer system). In vitro responses of both CD4$^+$ V$\beta_6{}^+$ and CD8$^+$ V$\beta_6{}^+$ cells to Mls-1^a were dependent upon the MHC haplotype of the stimulator cells, with I-E$^+$ (H-2^d or H-2^k) alleles being much more stimulatory than I-E$^-$ (H-2^q). These data strengthen the analogy between Mls gene products and other MHC class II-dependent superantigens such as the bacterial enterotoxins.

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