RESTRICTED TISSUE DISTRIBUTION OF
Mls^a DETERMINANTS

Stimulation of Mls^a-reactive T Cells by B Cells but not by Dendritic Cells or Macrophages

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Physiological responses of T cells are directed to fragments of exogenous antigen (antigen X) complexed to self MHC molecules (reviewed in reference 1). The frequency of T cells recognizing individual self + X epitopes is extremely low. T cells show higher responsiveness to allogeneic MHC molecules, with up to 3% of T cells responding to particular allo-MHC differences. In mice, T cells also give very strong responses to another set of alloantigens, termed Mls determinants (2). In fact the precursor frequency of murine T cells for Mls determinants is even higher than to allo-MHC (H-2) differences (3, 4). Although a number of Mls "alleles" have been described (2), particularly strong primary anti-Mls responses are directed to Mls^a determinants encoded on chromosome 1. Somewhat lower responses are elicited by Mls^c determinants, which have recently been shown to be encoded by a locus unlinked to the *Mls^a* locus (5).

As for responses to self + X and allo-H-2 epitopes, TCR-$\alpha\beta$ play an important role in controlling T cell responses to Mls^a determinants. The most direct evidence that anti-Mls^a responses are TCR dependent has come from the recent finding that T cell responsiveness to Mls^a determinants correlates with expression of particular TCR V$_\beta$ chains (6, 7). This finding has given rise to the idea that, like typical antigen X epitopes, Mls^a determinants are peptides that physically associate with H-2 molecules (6). Although this notion provides a satisfactory explanation for the TCR dependence of anti-Mls^a responses, it should be remembered that Mls^a determinants have a number of unusual properties. First, the precursor frequency of T cells for Mls^a determinants is far higher than to any known peptide (3, 4). Second, whereas most peptides elicit antibody production, Mls^a determinants have yet to be detected serologically. Third, in contrast to other cell-associated alloantigens, e.g., minor H antigens, Mls^a determinants fail to act as transplantation antigens or elicit graft-vs.-host reactions (8–10). Fourth, unlike responses to self + X epitopes, T cells generally recognize Mls^a determinants across H-2 barriers (11, 12). Fifth, in contrast to H-2 determinants, Mls^a determinants expressed on small resting B cells are strongly stimulatory for unprimed T cells (13). Finally, the response to Mls^a deter-

minants is unidirectional; whereas Mls^b T cells respond strongly to Mls^a stimulators, the response of Mls^a T cells to Mls^b stimulators is weak or absent.

In light of these unusual properties, we (14) and others (15) have suggested that Mls^a determinants are accessory molecules expressed on APC. According to this idea, T cells express a complementary Mls^a-reactive accessory molecule distinct from the TCR, the function of this molecule being to augment TCR contact with self H-2 (Ia) molecules on APC; T cell triggering occurs when the intrinsic affinity of the TCR for Ia molecules is above a certain threshold. Although this scenario is still highly speculative, it is of interest that a triple-reactive hybridoma responsive to self + X, allo H-2, and Mls^a determinants can lose reactivity to self + X and allo H-2 determinants but retain Mls^a reactivity, and vice versa (14). This finding implies that the recognition phase of Mls^a reactivity is not controlled solely by the TCR.

If Mls^a determinants fall under the category of "conventional" MHC-associated peptides, one would expect presentation of Mls^a determinants to T cells to be a property of any Ia$^+$ APC. The evidence on this point is conflicting. There are reports that B cells (13, 16–18), macrophages (MΦ)^1 (19–21), and dendritic cells (DC) (22) are all capable of inducing anti-Mls^a responses. However, one group observed that spleen cells from mice given anti-μ antiserum from birth to remove B cells were unable to stimulate anti-Mls^a responses (17). Since APC function for allo-H-2 responses was maintained, the results of this study are not in accord with the view that Ig$^-$ cells such as MΦ and DC can present Mls^a determinants. Therefore, the question arises as to whether the reports that MΦ and DC do stimulate anti-Mls^a responses could be attributed to minor contamination with B cells.

In this report we have used unprimed T cells and an Mls^a-specific T hybridoma to attempt to resolve the question of which cell types are capable of eliciting anti-Mls^a responses. The results confirm that spleen cells from μ-suppressed mice (μsm) are unable to stimulate anti-Mls^a responses. In addition, evidence is presented that, unlike B cells, preparations of MΦ and DC thoroughly depleted of B cells are very poor stimulators of Mls^a-reactive T cells. These findings suggest that very few cell types, perhaps only B cells, have the capacity to express Mls^a determinants in a stimulatory form. Since studies with B cells differentiating in bone marrow chimeras provided no evidence that Mls^a determinants can move from one cell to another, the data are difficult to reconcile with the view that Mls^a determinants are typical H-2-associated peptides.

Materials and Methods

Mice. B10.D2, B10.BR, C57BL/6 (B6), (B6 × CBA/J)F$_1$, AKR/J, DBA/2, and CBA/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. (B6 × CBA/Ca)F$_1$, B10.P, D1.LP, and some DBA/2 were obtained from the breeding colony of the Research Institute of Scripps Clinic, La Jolla, CA.

Anti-μ Suppression. Newborn mice were given daily intraperitoneal injections of affinity-purified rabbit anti-mouse μ antibodies (23) from day 0 to 5 (0.2 mg in 100 μl/injection).

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^{1} *Abbreviations used in this paper:* CAS, supernatant from Con A-stimulated spleen cells; DC, dendritic cells; FγG, fowl gamma globulin; LN, lymph node; MΦ, macrophages; μsm, μ-suppressed mice; PEC, peritoneal exudate cells; RPC, resident peritoneal cells.

Thereafter, the injections were given three times per week (0.4 mg in 200 μl/injection). In each experiment, μsm were checked for percentage of Ig^+ B cells by staining with FITC anti-Ig.

Chimeras. Mice were irradiated with a ^{137}Cs source delivered by a γ cell 40 irradiator (90 rad/min). Within 12 h of irradiation the mice were reconstituted with bone marrow cells pretreated with anti-Thy-1 mAb and C'. The mice were left for 5–8 wk before the experiment.

Production and Assay of T Hybridoma. The triple-reactive T clone used for fusion has been previously described (24). This B10.D2 clone reacts (*a*) to fowl gamma globulin (FγG) plus $H-2^d$, (*b*) to allo $H-2^{p,b,q}$, and (*c*) to Mls^a determinants; the clone recognizes Mls^a determinants in the context of all H-2 haplotypes tested including k, d, v, and u. Using standard techniques (14), the clone was fused to the $TCR-\alpha^-/\beta^-$ mutant of BW5147 derived by the Marrack/Kappler group. The resulting triple-reactive T hybridoma, KW.3, was maintained in DMEM (Gibco Laboratories, Grand Island, NY) containing 10% NCTC 109, 10% FCS, glutamine, 2-ME, and antibiotics. The specificity of the hybrid was assayed by stimulation of IL-2 production (14): 1–2 × 10^5 T hybrids were cultured with the indicated stimulator cells; 24 h later serial dilutions of the culture supernatants were added to 5 × 10^3 IL-2-dependent HT2 cells for an additional 24 h. The cultures were then pulsed with [^{3}H]thymidine for 16–18 h before harvest on glass fiber filters.

MLR. The culture media used for MLR was RPMI 1640 (Gibco Laboratories) containing 10% FCS, 5% NCTC 109, glutamine, 5 × 10^{-5} M 2-ME, and antibiotics. Indomethacin, a prostaglandin inhibitor, was included in the culture media at a concentration of 10^{-6} M as a precaution against nonspecific negative effects of macrophages. It was found to have a significant enhancing effect on the specific response in some experiments. 1–2 × 10^5 lymph node (LN) T cells were cultured with various concentrations of the indicated stimulators in flat-bottomed microtiter wells for 4 d; responses obtained at optimal concentrations of the indicated stimulators are shown. The cultures were pulsed with [^{3}H]thymidine during the last 18 h of culture. In most experiments $CD4^+$ T cells were enriched by incubating LN cells at 37°C for 1 h with C' plus a mixture of mAb reactive to B cells (J11d) and $CD8^+$ cells (3.168) (25). In some experiments, including the ones illustrated in Tables II and III, T cells were purified by passage over nylon wool columns before treatment with antibody + C'.

Preparation of DC. DC were prepared according to Steinman et al. (26). Briefly, spleen cells were allowed to adhere to plastic tissue culture dishes. After 2–3 h the plates were gently washed free of nonadherent cells and the adherent cells were incubated overnight. Nonadherent (floating) cells were then removed from the dishes and allowed to readhere for 1–2 h. The nonadherent cells (DC-enriched cells) were harvested. In some experiments these cells were further purified by treatment with J11d mAb + C' at 37°C for 1 h followed by washing; J11d mAb + C destroy nearly all Ig^+ B cells, neutrophils, and erythrocytes but spares T cells and the majority of MΦ and DC (27 and J. Sprent and M. Schaefer, unpublished data).

Preparation of Peritoneal Cells. Resident peritoneal cells were obtained from mice injected intraperitoneally 24 h before harvest with 1 ml of supernatant from Con A-stimulated spleen cells (CAS). For peritoneal exudate cells (PEC), mice were injected with 2.5 ml i.p. of a 3% sterile solution of thioglycollate medium. 2–3 d later these mice received 1 ml i.p. of CAS; PEC were collected 24 h later. Peritoneal cells were further purified by treating with J11d mAb + C' at 37° before culture.

Purification of B Cells. B cells were purified from pooled LN and spleens (13). After lysis of erythrocytes, the cells were incubated for 2 h on plastic (tissue culture 3003; Falcon Labware, Oxnard, CA) followed by two sequential passages through G10 columns. The resulting adherent cell–depleted suspension was further purified by incubation with a mixture of anti-Thy-1 (J1j) (27), anti-Mac 1 (M1/70) (13), and anti-DC (33D1) (28) mAb + C'. Such B cells are >99% IgM^+ and contain undetectable numbers of latex ingesting cells. For Exp. 2 of Table V, these B cells were further fractionated on Percoll density gradients as described (13) and only the less dense fraction was used. This fraction was previously shown to stimulate self + X and allo-H-2 responses as well as anti-Mls^a responses (13 and Webb, unpublished observation). Before use as stimulators, B cells were incubated with 25 μg/ml mitomycin C at 37°C for 30 min, followed by three washes.

Results

Experimental Design.   For measuring primary MLR, purified LN T cells or CD4$^+$ cells from mice of the nonstimulatory Mls^b allele were cultured with irradiated H-2-different stimulators vs. H-2-compatible Mls^a-positive stimulators; it should be noted that some Mls^a-positive strains, e.g., CBA/J, also express Mls^c determinants. In some experiments Mls^a determinants were detected with the aid of an Mls^a-reactive T hybridoma; this hybridoma also reacts to certain allo-H-2 determinants (H-2^{p,b,q}) and to self MHC/X epitopes (H-2^d plus FγG).

APC Function of Spleen Cells from B Cell–depleted μ-suppressed Mice.   At the outset of these experiments, we considered it of utmost importance to reassess the claim of Ahmed et al. (17) that lymphoid cells from μsm are unable to stimulate anti-Mls^a responses. To deplete mice of IgM$^+$ B cells, CBA/J (H-2^k, Mls^a) mice were given repeated injections of purified rabbit anti–mouse μ chain antibodies from birth (Materials and Methods). In accordance with previous findings (23), the spleens of these mice tested at 6 wk of age were essentially devoid of IgM$^+$ B cells. When used as a source of APC for primary MLR, Thy-1$^-$ spleen cells from CBA/J μsm failed to stimulate an anti-Mls^a response by H-2-compatible B10.BR (H-2^k, Mls^b) T cells (Table I). Normal Thy-1$^-$ CBA/J spleen cells, by contrast, were strongly stimulatory for B10.BR T cells. Although CBA/J μsm spleen cells failed to present Mls^a determinants, these spleen cells retained the capacity to stimulate anti-H-2 responses. Thus, when used as stimulators for H-2-incompatible B10.D2 (H-2^k, Mls^b) T cells, CBA/J μsm spleen cells elicited approximately the same response as B10.BR spleen cells.

The failure of μsm spleen cells to stimulate anti-Mls^a responses applied not only to CBA/J μsm (Table I) but also to DBA/2 (H-2^d, Mls^a) and D1.LP (H-2^b, Mls^a) μsm (data not shown). With all three strains, doses of Thy-1$^-$ μsm spleen cells ranging from 5 × 10^4 to 5 × 10^5 elicited virtually no anti-Mls^a response, but stimulated strong anti-H-2 responses. Suppression did not appear to be involved because

TABLE I

Primary MLR to Mls^a vs. Allo-H-2 Determinants: Failure of Spleen Cells from B Cell–depleted μsm to Stimulate Anti-Mls^a Responses

		[^{3}H]TdR Uptake responders*	
Thy-1$^-$ stimulators	(H-2,Mls)	B10.BR (H-2^k,Mls^b)	B10.D2 (H-2^d,Mls^b)
		cpm × 10^{-3}	
Normal B10.BR	(k,b)	1.3$^‖$	48.4
Normal B10.D2	(d,b)	34.3	2.1
Normal CBA/J	(k,a/c)	162.5	276.4
μsm CBA/J	(k,a/c) No. 1	2.4	58.3
	(k,a/c) No. 2	2.9	49.5

μsm were treated from birth with affinity-purified rabbit anti-μ antibody (Materials and Methods).
* 2 × 10^5 T cell–enriched responders (lymph node cells treated with Jlld mab + C') were cultured with 5 × 10^5 irradiated (1,500 rad) anti-Thy-1 + C'-treated spleen cells for 4 d. During the last 18 h each culture was pulsed with 1 μCi [^{3}H]TdR.
$^‖$ Mean of triplicate cultures.

adding μsm spleen to normal Mls^a-positive spleen cells failed to cause inhibition. The data thus confirm the finding of Ahmed et al. (17) that μsm spleen cells contain APC for anti-H-2 responses but lack APC for anti-Mls^a responses.

APC Function of DC. The above data are difficult to reconcile with reports that normal MΦ and DC elicit anti-Mls^a responses (see Introduction). One explanation for this discrepancy is that MΦ and DC in μsm do not make contact with B cells and so are unable to absorb Mls^a determinants from these cells. To examine this question, DC were prepared from spleens of normal mice and tested for their capacity to stimulate anti-Mls^a vs. anti-H-2 responses. To ensure that the anti-H-2 responses were directed to class II (Ia) determinants rather than to class I determinants, CD4$^+$-enriched T cells were used as responder cells (Materials and Methods).

In initial experiments, preparations of normal DC elicited very high anti-H-2 responses but also stimulated high anti-Mls^a responses. Since our DC preparations were found to contain Ig$^+$ cells, DC suspensions were depleted of B cells by treatment with J11d mAb + C. As shown in Table II, J11d$^-$ DC were strongly stimulatory for anti-H-2 responses. Thus, B10.BR CD4$^+$ T cells responded as effectively to J11d + C-treated B10.P (H-2^P) DC as to untreated B10.P DC. For anti-Mls^a responses, however, J11d$^-$ DC failed to stimulate. Thus, B10.BR CD4$^+$ cells responded well to untreated AKR/J DC but gave only background responses to J11d + C-treated AKR/J DC. A more comprehensive experiment with J11d$^-$ DC from four different strains is shown in Table III. It can be seen that, in contrast to Thy-1$^-$ spleen cells, J11d$^-$ DC from AKR/J and CBA/J mice failed to stimulate H-2-compatible Mls^a-reactive B10.BR CD4$^+$ cells. These same DC, however, were strongly stimulatory for H-2-different B10.D2 CD4$^+$ cells.

The inability of B-depleted DC to stimulate anti-Mls^a responses applied not only to unprimed CD4$^+$ responders but also to T hybridoma cells. Thus, as shown in Table III, the Mls^a-reactive T hybridoma responded well to Mls^a-positive CBA/J

TABLE II

Primary MLR Elicited by DC: J11d$^-$ DC Stimulate Anti-H-2 MLR but not Anti-Mls^a MLR

Stimulator strain	(H-2, Mls)	Stimulus	Treatment of DC	Proliferative response ([^{3}H]TdR uptake) of B10.BR CD4$^+$ T cells
				cpm × 10^{-3}
B10.BR	H-2^k, Mls^b	Self	–	10.5*
			J11d + C'	10.9
B10.P	H-2^P, Mls^b	H-2^P	–	71.5
			J11d + C'	73.5
AKR/J	H-2^k, Mls^a	Mls^a	–	131.5
			J11d + C'	8.1

Primary MLR were measured by culture of 2 × 10^5 CD4$^+$ T cells (Nylon wool-purified lymph node cell suspensions treated with J11d + anti-Lyt-2 mab + C') with either 5 × 10^5 Thy-1$^-$ splenic APC or 2.5 × 10^4 purified DC prepared from spleen (Materials and Methods); both stimulator populations were irradiated (1,500 rad). Cultures were incubated for 4 d and pulsed with [^{3}H]TdR during the last 18 h.
* Mean of triplicate cultures.

TABLE III

Anti-Mls^a Responses by Normal CD4$^+$ T Cells vs. T Hybridoma Cells: Responsiveness to Thy-1$^-$ Spleen Stimulators but not to Jlld$^-$ DC

	[^{3}H]TdR Uptake with stimulators									
	B10.BR (H-2^k,Mls^b)		CBA/J (H-2^k,Mls$^{a/c}$)		AKR/J (H-2^k,Mls^a)		B6 (H-2^b,Mls^b)		B10.D2 (H-2^d,Mls^b)	
Responders	Spleen	DC	Spleen	DC	Spleen	DC	Spleen	DC	Spleen	DC
					cpm × 10^{-3}					
T Hybridoma*	0.1§	0.6	48.8	0.2	39.3	0.4	33.9	28.0	ND	ND
B10.BR‡										
CD4$^+$ Cells	4.1	6.8	287.9	7.1	60.4	2.4	34.6	72.5	ND	ND
B10.D2										
CD4$^+$ cells	152.5	127.9	154.2	126.9	183.6	52.0	87.0	76.5	12.6	ND

* IL-2 production of T hybridoma in response to stimulators measured at 24 h; supernatants from primary cultures were incubated with the Il-2-sensitive indicator line HT-2. The hybridoma reacts to Mls^a determinants and also expresses alloreactivity for H-2^{p,b,q}.

‡ MLR were measured as for Table II; T cells were purified as in Table II. Spleen stimulators were treated with anti-Thy-1 mAb + C' and DC were treated with Jlld + C'; the stimulators were irradiated (1,500 rad) before use.

§ Mean of triplicate cultures.

and AKR/J spleen cells but was totally unresponsive to DC from these strains. As a manifestation of H-2 alloreactivity, the T hybridoma responded well to DC expressing H-2^b (B6) determinants (Table III).

The above data refer to DC preparations depleted of B cells by Jlld mAb + C treatment. In one experiment, mAb 14.8, specific for B220 molecules, which are expressed on all B cells (29), was used to fractionate AKR/J DC by FACS. The T hybridoma gave no detectable response to the B220$^-$ fraction of AKR/J DC, although these B220$^-$ cells were strongly stimulatory for H-2-reactive DBA/2 T cells in primary MLR (data not shown).

APC Function of MΦ.   Cell populations enriched for MΦ were obtained from the peritoneal cavity of normal mice or mice given thioglycollate 3 d before. To enhance Ia expression, the MΦ donors were given CAS intraperitonally 1 d before harvest. Before use as stimulators, peritoneal cell preparations were pretreated with Jlld mAb + C to remove Ig$^+$ cells. In preliminary experiments, resident peritoneal cells (RPC) and PEC were tested for their capacity to stimulate primary anti-H-2 MLR, using normal CD4$^+$ cells as responders. With both MΦ populations, primary MLR were negligible, even in the presence of indomethacin and over a wide dose range of stimulators (data not shown). With T hybridoma cells, however, MΦ were clearly able to present allo-H-2 (H-2^b) determinants (Table IV). Thus, in addition to reacting to B6 spleen and DC, the T hybridoma responded well to B6 RPC and PEC. Significantly, this hybridoma gave no detectable response to AKR/J RPC or PEC, i.e., cells of Mls^a genotype (Table IV); strong responses were observed with AKR/J spleen cells. Thus, like DC, peritoneal MΦ presented H-2 (Ia) alloantigens but failed to present Mls^a determinants.

APC Function of Purified B Cells.   As discussed earlier (see Introduction), resting T cells give strong anti-Mls^a responses to purified B cells (13). This is illustrated in Table V. The responses of T hybridoma cells to highly purified B cells as APC

TABLE IV

APC Function of CAS-induced Mɸ Populations for T Hybridoma:
Mɸ Present Allo-H-2 (H-2^b) Determinants but not Mls^a Determinants

Stimulators		No. of stimulators for T hybridomas	[^{3}H]TdR Uptake by HT-2 cells[‡]
Strain	Cell type[*]		
			cpm × 10^{-3}
B10.BR	Spleen (Thy 1$^-$)	5 × 10^5	0.1[§]
(H-2^k,Mls^b)	TG-PEC (Jlld$^-$)	5 × 10^4	0.1
	(RPC (Jlld$^-$)	2 × 10^4	0.1
	DC (Jlld$^-$)	2 × 10^4	0.1
AKR/J	Spleen (Thy 1$^-$)	5 × 10^5	40.7
(H-2^k,Mls^a)	TG-PEC (Jlld$^-$)	5 × 10^4	0.1
		10^4	0.1
	RPC (Jlld$^-$)	2 × 10^4	0.3
		10^4	0.1
	DC (Jlld$^-$)	2 × 10^4	0.2
B6	Spleen (Thy 1$^-$)	5 × 10^5	31.7
(H-2^b,Mls^b)	TG-PEC (Jlld$^-$)	5 × 10^4	14.8
		2.5 × 10^4	4.2
		10^4	0.4
	RPC (Jlld$^-$)	2 × 10^4	38.7
		10^4	11.8
	DC (Jlld$^-$)	2 × 10^4	35.4

[*] Spleen cells were treated with anti-Thy-1 mab + C'. Thioglycollate-induced PEC were from mice that received 2.5 ml i.p. of 3% thioglycollate at day − 3 and 1 ml i.p. of CAS on day − 1 (Materials and Methods). RPC were from mice that had received 1 ml i.p. CAS at day − 1. All Mɸ and DC preparations were treated with Jlld + C' to remove B cells and were then irradiated (1,500 rad).

[‡] IL-2 production by T hybridoma; see Table III.

[§] Mean of triplicate cultures.

is also shown; B cells were prepared from spleen and LN by removal of adherent cells on plastic followed by passage on G10 and treatment with anti-Thy-1, 33D1, and C'. It can be seen that B cells were highly effective APC for each of the three ligands recognized by the hybridoma, i.e., FγG + self H-2^d (B10.D2), allo-H-2^P(B10.P), and Mls^a (CBA/J).

Can B Cells Absorb Mls^a Determinants from other B Cells In Vivo? One explanation for the failure of DC and Mɸ to express Mls^a determinants is that these determinants are incapable of moving in intact form from one cell to another. To address this question, we examined whether Mls^a-negative B cells, i.e., B6 B cells, could absorb Mls^a determinants in a chimeric enviroment. The experimental system used here was to transfer a mixture of T-depleted B6 (H-2^b, Mls^b) and CBA/J (H-2^k, Mls^a) bone marrow cells into heavily-irradiated (1,100 rad) (B6 × CBA/Ca [H-2^b, Mls^b × H-2^k, Mls^b])F$_1$ mice (see Table VI, *footnote*). The recipients were left for 5–8 wk to allow the formation of a mixed population of B6 and CBA/J B cells. Would the B6 B cells absorb Mls^a determinants from the CBA/J B cells? This question was addressed by fractionating the spleen cells into B6 (H-2^b) and CBA/J (H-2^k) B cell–enriched populations with the aid of appropriate anti-H-2 alloantibody mAb plus C' (in addition to anti-Thy-1 mAb).

TABLE V

APC Function of Purified B Cells for T Hybridoma Cells

			[^{3}H]TdR Uptake with stimulators				
Exp.	Responders*	APC‡	B10.D2 (H-2^d,Mls^b)	B10.D2/FγG	B10.BR (H-2^k,Mls^b)	CBA/J (H-2^k,Mls$^{a/c}$)	B10.P (H-2^p,Mls^b)
					cpm × 10^{-3}§		
1	B10.BR CD4$^+$	Spleen	–	–	2.6	276.2	111.2
		B cells	–	–	0.4	280.2	32.3
	T hybridoma	Spleen	–	–	0.4	49.8	49.2
		B cells	–	–	0.5	47.6	48.4
2	T hybridoma	Spleen	0.2	18.5	–	56.9	40.6
		B cells	0.4	18.8	–	58.4	47.6

* B10.BR CD4$^+$ responder cells (2 × 10^5/culture) were prepared by passing LN cells through nylon wool columns and then treating the recovered cells with anti-CD8 mAb + C.

‡ Spleen stimulators were pretreated with anti-Thy-1 mAb + C. Purified B stimulators were prepared from pooled spleen + LN as described in Materials and Methods. For Exp. 2 (but not Exp. 1), the purified B cells were separated on Percoll gradients to obtain a fraction of low-density B cells; in our hands (13 and Webb, unpublished results), these latter cells are more effective than typical high-density B cells in presenting self + X (FγG) epitopes. All stimulator cells were used at a dose of 5 × 10^5/culure and pretreated with mitomycin C (not irradiation).

§ For the MLR by CD4$^+$ cells, responses were measured on day 4 as for Table II. For the response of the T hybridoma, IL-2 production was measured as for Table III.

TABLE VI

Anti-Mls^a MLR Elicited by Thy-1$^-$ Spleen Cells from B6 + CBA/J → 1,100-rad

(B6 × CBA/Ca)F$_1$ Chimeras: no Stimulation with Anti-I-A^k + C-treated Spleen Cells

	Stimulators			[^{3}H]TdR Uptake responders		
Exp.	Strain	H-2,Mls	Treatment	B6 (H-2^b,Mls^b)	(B6 × CBA/Ca)F$_1$ (H-2^b,Mls^b × H-2^k,Mls^b)	B10.BR (H-2^k,Mls^b)
					cpm × 10^{-3}	
1	B6	b,b	–	6.3	3.4	30.7
	(B6 × CBA/Ca)F$_1$	bxk,bxb	–	31.8	3.5	29.0
	D1.LP	b,a	–	50.9	73.1	127.8
	(B6 × CBA/J)F$_1$	bxk,bxa/c	–	97.2	100.4	98.0
	Chimera 1		–	54.8	65.4	68.9
			Anti-I-A^b + C	47.9	89.0	23.4
			Anti-I-A^k + C'	2.2	2.0	20.0
2	Chimera 2		–	48.3	52.9	34.8
			Anti-I-A^k + C'	1.8	0.9	15.3
	Chimera 3		–	118.8	81.6	126.5
			Anti-I-A^k + C'	1.5	0.7	21.9
	Chimera 4		–	61.2	26.6	47.3
			Anti-I-A^k + C	2.9	1.7	44.7

(B6 × CBA/Ca)F$_1$ mice were irradiated (1,100 rad) and reconstituted intravenously with a mixture of T-depleted B6 marrow (10^7/mouse) and CBA/J marrow (5 × 10^6/mouse). Higher numbers of B6 marrow cells were given to overcome Hh resistance. At 5 wk (Exp. 1) or 8 wk (Exp. 2) after reconstitution, the chimeras were tested for Mls^a-specific APC function. Doses of 2 × 10^5 CD4$^+$ responder cells (Table III) were cultured for 4 d with 5 × 10^5 irradiated (1,500 rad) Thy-1$^-$ spleen cells for 4 d and pulsed with [^{3}H]TdR during the last 18 h of culture. Where indicated, the chimera spleen stimulators were pretreated with either anti-I-A^k (11.5.2) + anti-Thy-1 mab + C' or with anti-I-A^b (28.16.8s) + anti-Thy-1 mab + C' (30). All stimulators were washed four times before culture.

The results of this experiment are shown in Table VI. Unseparated spleen cells from the chimeras, as well as anti-I-A^b + C–treated spleen cells, were strongly stimulatory for (B6 × CBA/Ca)F$_1$ T cells, i.e., for Mls^a-reactive cells. Significantly, however, removing CBA/J cells from the spleen suspensions with anti-I-A^k mAb + C (which destroyed only ∼50% of the spleen cells) completely removed the capacity to stimulate anti-Mls^a responses. Thus, the B6 cells surviving anti-I-A^k + C treatment were totally nonstimulatory for Mls^a-reactive syngeneic B6 and semisyngeneic (B6 × CBA/Ca)F$_1$ T cells but retained the capacity to stimulate H-2-different B10.BR T cells. These findings thus provided evidence that B6 cells cannot absorb (and/or process) Mls^a determinants from CBA/J cells during prolonged cell-to-cell contact in vivo.

Discussion

Although various Ia$^+$ cell populations can present antigen to sensitized T cells, APC function for unprimed T cells seems to be largely restricted to DC (31). The decisive influence of DC on the function of unprimed T cells applies to typical self H-2/X responses as well as to anti-H-2 MLR. Responses to Mls^a determinants, however, seem to be exceptional in two respects. First, as mentioned earlier (see Introduction), strong primary MLR to Mls^a determinants can be elicited by highly purified populations of normal B cells (13). Second, the data presented in this paper suggest that B cell–depleted populations, including purified DC, fail to elicit anti-Mls^a responses.

The finding that B-depleted μsm spleen cells, as well as J11d$^-$ DC and MΦ from normal mice, were all unable to stimulate anti-Mls^a responses raises the possibility that Mls^a determinants are expressed solely on B cells. Although the data are in accord with this notion, the possibility that Mls^a determinants are expressed on a minor subset of Ig$^-$ cells cannot be ruled out. It is also conceivable that some cells express Mls^a determinants in nonstimulatory form. Thus, given that anti-Mls^a responses appear to require corecognition of Ia molecules (3, 6, 7), expression of Mls^a determinants on Ia$^-$ cells would presumably go undetected. In this respect it should be mentioned that T cells from μsm show at least partial tolerance to "self" Mls^a determinants (Webb, unpublished results). Whether this finding reflects the expression of Mls^a determinants on non-B cells or has some other explanation (e.g., homing of small numbers of Ig$^+$ cells to the thymus prenatally) is still unclear.

The failure to find convincing evidence of Mls^a expression by non-B APC makes it difficult to argue that Mls^a determinants represent typical MHC-associated peptides. It is also noteworthy that Mls^a-negative B cells did not absorb detectable levels of Mls^a determinants in appropriate bone marrow chimeras (Table VI). This finding contrasts with reports that certain other H-2-associated alloantigens, e.g., minor H antigens (32) and β$_2$-microglobulin (33), do move freely from one cell to another. The data also contrast with the observation of Marrack and Kappler (34) that an allo I-E-associated epitope recognized by Vβ17a$^+$ T cells is expressed on B cells and fresh MΦ (RPC) but is not found on cultured MΦ; the authors suggested that the I-E-associated epitope is B cell derived but can be absorbed by MΦ and displayed in processed form. Since we failed to detect Mls^a determinants on fresh MΦ, it would appear that Mls^a determinants are fundamentally different from the I-E-associated epitope studied by Marrack and Kappler (34).

Two other models for Mls^a recognition require consideration. First, Mls^a determinants on B cells might be expressed on a cell surface molecule that associates with H-2 (Ia) molecules, but without processing (breakdown into peptides) (data not shown). T cells could then recognize this complex via a single TCR combining site. This model is compatible with much of the available data (e.g., references 6 and 7), but it fails to explain our finding that anti-Mls^a reactivity segregates independently from self + X and allo H-2 reactivity in T hybridomas (14). For this reason we favor a model in which the recognition phase of anti-Mls^a responses involves an extra T cell molecule (which we envisage as an accessory molecule). For this model it is not necessary to postulate that Mls^a determinants enter into a physical association with Ia molecules.

Until Mls^a determinants are characterized biochemically, no model for Mls^a recognition by T cells can be put forward with any degree of confidence. The data in this paper do not clarify the physiological status of Mls^a determinants. However, the data do suggest strongly that Mls^a determinants are highly unusual and do not fit easily into the category of typical self + X epitopes.

Summary

Evidence was sought on the tissue distribution of Mls^a determinants, a class of cell-associated non-H-2 alloantigens that is highly immunogenic for unprimed T cells. Whereas normal CD4$^+$ T cells and an Mls^a-reactive T hybridoma gave strong responses to Mls^a-positive stimulator populations containing Ig$^+$ B cells, anti-Mls^a responses to B-depleted stimulators were almost undetectable. The B-depleted stimulators tested included Thy-1$^-$ spleen cells from μ-suppressed mice (mice treated with anti-μ antibody from birth) and J11d$^-$ preparations of spleen dendritic cells (DC) and peritoneal macrophages (MΦ) from normal mice. Each of these populations was strongly immunogenic for allo-H-2-reactive T cells.

The failure to detect Mls^a determinants on Ig$^-$ APC, i.e., MΦ and DC, suggests that Mls^a determinants are not typical H-2-associated peptides. The data are more compatible with a model in which Mls^a determinants represent (or form part of) an integral cell membrane molecule expressed largely, and perhaps exclusively, on B cells. T cells might recognize these molecules only in native form, "processed" Mls^a determinants being nonimmunogenic. Consistent with this possibility, no evidence was found that Mls^a-negative B cells could absorb Mls^a determinants from Mls^a-positive B cells in a chimeric environment.

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