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Allelic Polymorphisms at the H-2A and HLA-DQ Loci Influence the Response of Murine Lymphocytes to the *Mycoplasma arthritidis* Superantigen MAM

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***Mycoplasma arthritidis*, an agent of rodent arthritis, produces a potent superantigen (SAg), MAM. Previous work established that MAM is presented to T cells by murine H-2E or the homologous human HLA-DR molecules and that lymphocytes lacking a functional H-2E molecule fail to respond to MAM. Recently, more potent and purified preparations of MAM of known protein content have become available. This enabled us to more effectively compare the response of MAM with that of other SAgs by using lymphocytes from mice whose cells express different H-2A and HLA-DQ molecules. Here we demonstrate that cells from some H-2E-negative mouse strains respond to higher concentrations of MAM. By use of inbred, congenic, and recombinant mice, we show that these differences are, in fact, exercised at the level of the major histocompatibility complex (MHC) and that allelic polymorphisms at H-2A influence reactivity to MAM. In addition, polymorphisms at HLA-DQ, the human homolog of H-2A, also influence responsiveness to MAM. Cells expressing DQw6 (HLA-DQA1*0103 and DQB1*0601 chains) gave much higher responses to MAM than did cells expressing DQw8 (DQA1*0301 and DQB1*0302 chains). In fact, responses of lymphocytes expressing DQB1*0601 chains homozygously were as high as those observed for cells expressing a functional H-2E molecule. Murine lymphocytes responded less well to staphylococcal enterotoxin B (SEB) and SEA, but mouse cells expressing human MHC molecules gave much higher responses. The patterns of reactivity observed with cells expressing the various murine and human alleles differed for MAM, SEB, and SEA, suggesting that each of these SAgs interacts with different regions or residues on MHC molecules. It has been hypothesized that SAgs might play a role in susceptibility to autoimmune disease. Allelic polymorphisms at MHC loci might therefore influence susceptibility to autoimmune disease by affecting immunoreactivity to specific superantigens.**

Superantigens (SAgs) activate T cells by cross-linking major histocompatibility complex (MHC) class II molecules on accessory cell surfaces with the V_β chains of the T-cell receptor (TCR) for antigen. Unlike classic native antigens, SAgs do not require processing (11) since they bind directly to MHC molecules (22, 24, 42, 51, 52) at sites outside of the antigen groove (19). Each SAg not only shows specificity for its particular panel of V_β chain TCRs (13, 14, 40) but may also exhibit specific binding to different isotypes, regions, or residues of class II molecules (26, 28, 31, 53).

Recently, alternate pathways of T-cell activation by SAgs have been found. For example, class I molecules can play a role in presenting SAgs to T cells (27), and T-cell activation in the absence of MHC molecules has also been described (20). Furthermore, MHC-associated peptides can influence the binding of SAg to MHC molecules (6, 58).

Class I and class II MHC molecules are important to antigen and SAg activation of the immune system. Both are heterodimeric membrane-associated molecules composed of alpha and beta protein subunits. In the case of the class I molecules, the beta chain is always β_2-microglobulin and the alpha chain is encoded within the MHC region, whereas for class II molecules, both the alpha and beta chains are encoded within the MHC region and are typically inherited together as a haplotype. In mice, there are two class II loci, namely, those encoding the H-2A and H-2E proteins, which are the equivalents of the human HLA-DQ and HLA-DR molecules, respectively. For inbred mice, the MHC haplotypes are abbreviated with letters. For example, *H-2*^{k} mice have *k* haplotype genes coding for A_α, A_β, E_α, and E_β proteins. For H-2A and the related HLA-DQ molecules, both the alpha and beta chains are polymorphic, whereas H-2E and its homolog HLA-DR have highly conserved, similar, alpha chains but polymorphic beta chains. When one chain of a heterodimer is missing, hybrid molecules such as $E_\alpha A_\beta$, or $A_\alpha DQ_\beta$ in the case of mice transgenic for DQ_β genes, can occur (39).

A characteristic feature of the SAg MAM is its use of the H-2E molecule for presentation to T cells. In fact, one of the unique properties of MAM which originally led to its detection was its ability to activate T lymphocytes only from mouse strains which express H-2E (8). The earliest evidence that the conserved E_α chain bore the MAM receptor was that lymphocytes from E_β-negative, E_α-positive A.TFR5 mice, which express the hybrid $E_\alpha{}^k A_\beta{}^f$ molecule, responded to MAM and also absorbed the proliferative activity of MAM from solution (9). Subsequent studies using glass beads coated with liposomes with incorporated H-2E and H-2A molecules (4) and fibroblasts transfected with various H-2E, H-2A, and H-2EA hybrid molecules (12) indicated that the E_α chain alone or E_α in combination with E_β or A_β could effectively present MAM. In addition, lymphocytes from nonresponding H-2E-negative C57BL/10 or ACA mice, made transgenic for E_α, were found to respond fully to MAM. Lymphocytes from mice transgenic

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TABLE 1. Characterization of mouse strains used

Mouse strain(s)	MHC class I region haplotype				TCR V_β haplotype
	A_α	A_β	E_α[a]	E_β[b]	
BALB/c, B10.D2	*d*	*d*	*d* (+)	*d* (+)	*b*
B10.RDD	*d*	*d*	*b* (−)	*d* (+)	*b*
CBA, C3H/HeJ, B10.BR	*k*	*k*	*k* (+)	*k* (+)	*b*
B10.RSF2, B10.RKB	*k*	*k*	*b* (−)	*k* (+)	*b*
C57BL/6, C57BL/10	*b*	*b*	*b* (−)	*b* (+)	*b*
P/J, B10.P	*p*	*p*	*p* (+)	*p* (+)	*b*
B10.RPF1	*p*	*p*	*f* (−)	*p* (−)	*b*
SWR, B10.Q	*q*	*q*	*q* (−)	*q* (−)	*a*, *b*[c]
SJL, B10.S	*s*	*s*	*s* (−)	*s* (+)	*a*, *b*[c]
B10.M, B10.RFD6	*f*	*f*	*f* (−)	*f* (−)	*b*
B10.RFR	*f*	*f*	*s* (−)	*s* (+)	*b*

[a] The presence (+) or absence (−) of E_α expression is indicated in parentheses.
[b] The presence (+) or absence (−) of E_β expression is indicated in parentheses.
[c] $V_\beta{}^a$ SWR and SJL mice lack the V_β8 TCRs.

for the human HLA-DRA1 (DR_α) molecule could also respond to MAM (3), indicating that $DR_\alpha A_\beta$ hybrid molecules can function to present MAM to T cells.

Recently, a new purification procedure resulted in the isolation of small amounts of homogeneous MAM (2). The availability of these new potent preparations of known MAM protein content enabled us to reevaluate the interaction of MAM with murine and human class II molecules and to compare these responses with those observed for staphylococcal SAgs. In the present communication, we document that in the absence of H-2E, some murine H-2A molecules can present MAM to T cells and that polymorphisms at the locus encoding the H-2A protein and at the locus encoding the human equivalent, HLA-DQ, influence lymphocyte proliferation induced by MAM.

MATERIALS AND METHODS

Mouse strains used. Inbred mouse strains BALB/c, CBA/J, C3H/HeJ, C57BL/6, C57BL/10, P/J, SWR, and SJL were obtained from Jackson Laboratories (Bar Harbor, Maine). MHC congenic mouse strains on a C57BL/10 background, i.e., B10.D2, B10.BR, B10.P, B10.Q, B10.S, and B10.RIII, were also obtained from Jackson Laboratories. Congenic and recombinant strains B10.M, B10.RDD, B10.RSF2, B10.RKB, B10.RPF1, B10.RFD6, and B10.RFR were bred in the laboratories of Chella S. David. The haplotypes expressed at the H-2A and H-2E regions of the murine MHC and the TCR V_β chain haplotypes of these mice are listed in Table 1.

The B10.M mouse strain which is totally nonresponsive to MAM was used as a recipient for human MHC transgenes. Mice, heterozygous or homozygous for the locus encoding the HLA-DQ protein, were made by use of the HLA-DQB1*0601 transgene (abbreviated to DQ_βw6). Double-transgenic mice were made by use of the HLA-DQA1*0103/HLA-DQB1*0601 ($DQ_{\alpha/\beta}$w6) genes or HLA-DQA1*0301/HLA-DQB1*0302 ($DQ_{\alpha/\beta}$w8) genes in knockout mice lacking endogenous mouse class II molecules.

SAgs. The mycoplasma SAg MAM was prepared by a new procedure described previously (2). In brief, *Mycoplasma arthritidis* cultures in modified Hayflick broth were fractionated with $(NH_4)_2SO_4$, and the 50 to 80% precipitate was subjected to G-50 column chromatography followed by Sepharose S and Mono S exchange chromatographies. The resulting material contained about 10^7 U of activity as defined previously (1) and represented at least a 10^5-fold purification. MAM, at a dilution of 1:100, was stabilized by storage in aliquots in RPMI complete medium (see below) at −70°C. Since homogeneous MAM subsequently became available, we calculated that the undiluted preparations used in most of the experiments described here contained approximately 10 μg of pure MAM per ml. Maximal stimulation with H-2E-bearing mouse strains occurred in the range of 0.4 to 2 ng/ml. The SAgs Staphylococcal enterotoxin A (SEA) and B (SEB) were purchased from Toxin Technology (Madison, Wis.) and used at the concentrations indicated.

Lymphocyte proliferation assay. Uptake of [^{3}H]thymidine ([^{3}H]TdR) was used as a measure of lymphocyte activation as detailed previously (10). In brief, 5×10^5 cells/well, in triplicate wells, were suspended with inducers in 0.2 ml of RPMI complete medium, consisting of RPMI 1640 medium supplemented with 2 mM L-glutamine, 5% human serum, and 100 U of penicillin G per ml. Cultures were incubated at 37°C in 5% CO_2 in air for 48 h, pulsed for 24 h with 1 mCi of [^{3}H]TdR (2 Ci/mmol), and harvested at 72 h with a Skatron Basic 96 cell harvester. Filter mats were counted on a Skatron beta counter. The results were expressed as the mean counts per minute ± standard deviations obtained in lymphocyte cultures with inducers minus counts obtained without inducers.

Antibody inhibition of proliferation. Protein A-purified monoclonal antibody (MAb) to $A_\alpha{}^f$ was prepared in one of our laboratories (C. S. David). The purified MAb to DQ_βw6 (DQB1*0601 chain, 61.11.1) was a generous gift from H. Inoko (Tokai University School of Medicine, Ischara, Kanagawa, Japan). MAb to $E_\alpha{}^k$ (14.4.4S) was prepared and used as ascites fluid. To test for inhibition of proliferative responses to MAM, the antibody preparations were incubated for 1 h at 37°C with splenocytes prior to the addition of MAM.

RESULTS

Lymphocyte responses to MAM in H-2E-negative mice. Previous studies indicate that MAM interacts with the alpha chain of the murine H-2E and the human HLA-DR molecules and that lymphocytes from mouse strains lacking E_α fail to respond significantly to MAM. By employing new, potent MAM preparations, we recently obtained evidence that MAM can weakly activate lymphocytes from H-2E-negative C57BL/10 mice. In this study, we reevaluated the ability of MAM to activate lymphocytes lacking intact H-2E molecules. Details of the class II MHC molecules tested are shown in Table 1.

Four experiments were conducted to compare the proliferative responses to MAM of lymphocytes from E_α-positive BALB/c (*H-2*^{d}) mice with those from E_α-negative C57BL/6 and C57BL/10 mice; the responses to SEB and SEA were also tested. MAM elicited high responses in BALB/c splenocytes, with maximal responses at 2 ng/ml and responses still present at 16 pg/ml (Fig. 1). MAM also activated splenocytes from C57BL/10 and C57BL/6 mice but to a lesser degree, at dilutions of 10 and 2 ng/ml. Responses to SEB (Fig. 1) and SEA (data not shown) were strikingly less than those to MAM for all three mouse strains tested and required at least a 3-log increase in concentration for comparable counts to be achieved. We next compared the reactivities of lymphocytes from C3H (*H-2*^{k}) and congenic C3H.SW (*H-2*^{b}) mice to MAM and SEB. The data (Fig. 2) indicate that the weaker responses observed with lymphocytes from *H-2*^{b} mice are controlled by the MHC and are likely due to a lack of H-2E expression. Responses to SEB were also markedly lower.

We next surveyed other inbred mouse strains that were

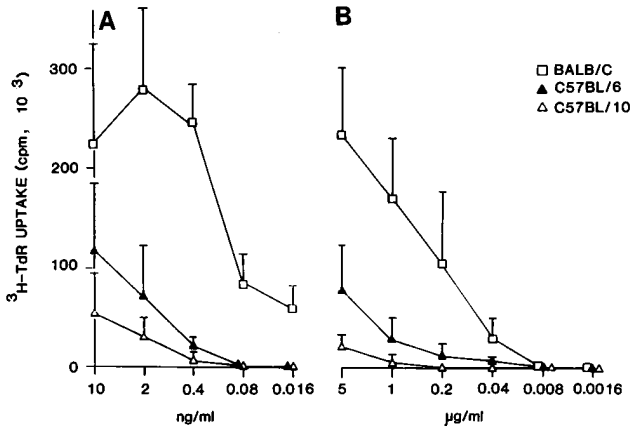

FIG. 1. Proliferative responses of lymphocytes from inbred mouse strains BALB/c ($E_\alpha{}^+$ *H-2*^{d}), C57BL/6 ($E_\alpha{}^-$ *H-2*^{b}), and C57BL/10 ($E_\alpha{}^-$ *H-2*^{b}) to MAM (A) and SEB (B). Data are expressed as uptake of [^{3}H]TdR in the presence of inducer minus uptake without inducer. Mean values ± standard deviations of triplicate determinations per mouse with at least three separate mice per strain are shown. Mice used possess the MAM-reactive TCR $V_\beta{}^b$ haplotype. Doses for MAM are given in nanograms per milliliter; doses for SEB are in micrograms per milliliter.

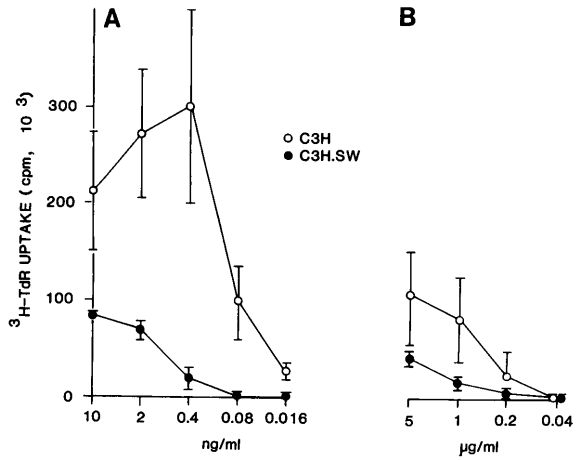

FIG. 2. Proliferative responses of lymphocytes from inbred mouse strain C3H (E_α^+ *H-2*^{k}) and congenic mouse strain C3H.SW (E_α^- *H-2*^{b}) to MAM (A) and SEB (B). See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

negative for H-2E and their H-2 congenic counterparts against a C57BL/10 background (Table 2). Lymphocytes from inbred SWR (*H-2*^{q}) and SJL (*H-2*^{s}) mice failed to respond to MAM irrespective of the concentration used. This lack of response could not be attributed to the lack of the V_β8 TCRs in these strains (Table 1) since lymphocytes from the congenic B10.Q and B10.S mice which express these and other MAM-reactive V_β chains also give negative responses to MAM. Lymphocytes from the congenic B10.M mice (*H-2*^{f}) also totally failed to respond to MAM as did cells from recombinant B10.RFD6 and B10.RFR mice, both of which express H-2^f. SEB and SEA induced positive responses in lymphocytes from all inbred and congenic strains, although higher concentrations were required for these SAgs than for MAM as is described above. The higher response to SEB observed for lymphocytes from the congenic strains compared to that of the inbred strains expressing H-2^q and H-2^s might be explained by the more limited T-cell repertoire (V_β^a haplotype) of SWR and SJL mice which lack the V_β8 TCRs that can be used by SEB. MAM totally failed to stimulate H-2^f-bearing lymphocytes, whereas SEA and SEB gave moderately high responses, in some cases at doses as low as 8 ng/ml.

The results presented indicate that there are differences in the abilities of mice of different MHC haplotypes to respond to MAM and that responses can be obtained in the absence of H-2E expression. In the following experiments, we examined whether these differences are due to polymorphisms at the H-2A gene.

Influence of polymorphisms at the H-2A locus. In the next experiment, we compared lymphocytes from inbred, H-2E$^+$, B10 congenic H-2E$^+$, and recombinant H-2E$^-$ mouse strains for responses to MAM and SEB. In Fig. 3, we show that splenocytes from CBA (*H-2*^{k}) mice respond highly to MAM, those from B10.BR (*H-2*^{k}) respond moderately to MAM, and those from recombinant B10.RKB and B10.RSF2 mice, which are *H-2A*^{k} H-2E$^-$, totally fail to respond to MAM. In contrast, SEB induced weak to moderate lymphocyte responses in all of these strains. Interestingly, the highest response to SEB was with H-2E$^-$ B10.RKB splenocytes, which totally failed to respond to MAM.

In Fig. 4, we show that lymphocytes from BALB/c (*H-2*^{d}) and congenic B10.D2 (*H-2*^{d}) mice respond highly or moderately to MAM, confirming previous observations, whereas those from recombinant B10.RDD mice (*H-2A*^{d} E_α^-) give a lesser response and only at 10 and 2 ng/ml. Whereas B10.RDD lymphocytes virtually failed to respond to SEB, those from B10.D2 and BALB/c mice responded significantly but only in the microgram range. Similar experiments using mouse cells expressing H-2^p (Fig. 5) revealed that P/J and B10.P lymphocytes responded well to MAM whereas the E_α^- B10.RPR$_1$ gave lesser responses at 10 and 2 ng/ml. All strains responded to SEB when it was used at very high concentrations. The combined data from Table 2 and Fig. 1 to 5 suggest that MAM can be presented by H-2A^b, H-2A^d, and H-2A^p but not by H-2A^q, H-2A^s, H-2A^f, or H-2A^k molecules. It is also apparent that in H-2E-negative strains, responsiveness to MAM does not coincide with responsiveness to SEB.

To confirm the role of A_α molecules in the presentation of MAM to T cells, we examined the effect of antibodies to class II molecules on the responses of lymphocytes to MAM (Table 3). Antibodies to H-2A^d, a presenter of MAM, as demonstrated above, inhibited the responses of *H-2A*^{d} E_α^- lymphocytes to MAM but had no effect on E_α^+ B10.BR cells. Antibodies to cells bearing H-2A^k, a MAM-nonresponsive haplotype, had no effect.

Presentation of MAM by HLA-DQ molecules. These studies were conducted to determine whether various HLA-DQ (the human equivalent of murine H-2A) transgenes could alter the nonresponsiveness of lymphocytes from E_α^- A_α^f B10.M mice to MAM. We chose the human DQ_βw6 (DQB1*0601) gene for testing due to its ability to mimic certain attributes of the murine E_α in deleting specific V_β TCRs in mice expressing retrovirus-encoded SAgs (52). First, we compared the re-

TABLE 2. Proliferative responses to MAM, SEA, and SEB by lymphocytes lacking H-2E$_\alpha$

Mouse strain	*H-2* haplotype	Specific uptake[a] of [^{3}H]TdR (cpm, 10^3) in response to:								
		MAM at concn (ng/ml) of:			SEB at concn (µg/ml) of:			SEA at concn (µg/ml) of:		
		10	0.4	0.0016	5	0.2	0.008	5	0.2	0.008
BALB/c (4)[b]	*d*	179.2	223.2	57.8	203.1	63.5	2.3	96.7	47.4	6.4
SWR (5)	*q*	1.5	0.4	0.3	22.5	2.2	0.7	53.4	17.6	8.3
B10.Q (4)	*q*	2.8	0.1	0	45.0	20.2	1.2	92.0	81.0	74.8
SJL (4)	*s*	3.3	0.8	1.2	25.1	4.2	2.5	28.0	20.0	8.4
B10.S (4)	*s*	1.7	0.1	0	91.0	44.2	5.7	105.9	112.2	99.8
B10.M (6)	*f*	2.3	0.2	0	152.9	63.8	4.1	93.0	87.2	80.8
B10.RFD6 (4)	*f*	1.4	0	0	46.2	7.7	0.3	66.3	63.7	18.5
B10.RFR (4)	*f*	1.4	0	0	166.0	129.9	40.6	123.8	116.2	65.6

[a] Specific uptake of [^{3}H]TdR is uptake in the presence of inducer minus uptake by cells alone.
[b] Values in parentheses are numbers of mice tested.

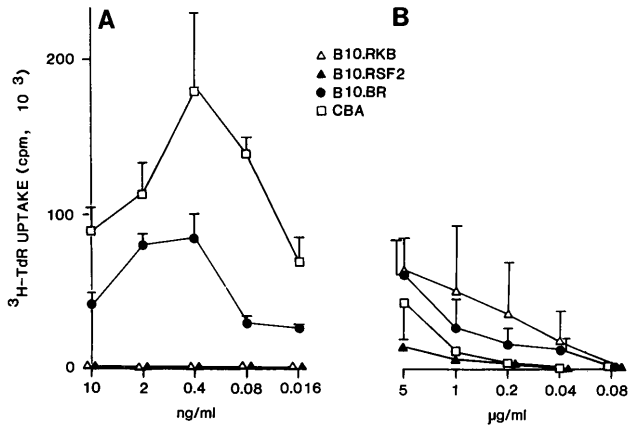

FIG. 3. Proliferative responses of lymphocytes from inbred mouse strain CBA (E_α^+ *H-2*^{k}), congenic mouse strain B10.BR (E_α^+ *H-2*^{k}), and recombinant mouse strains B10.RKB (E_α^- *H-2A*^{k}) and B10.RSF2 (E_α^- *H-2A*^{k}) to MAM (A) and SEB (B). See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

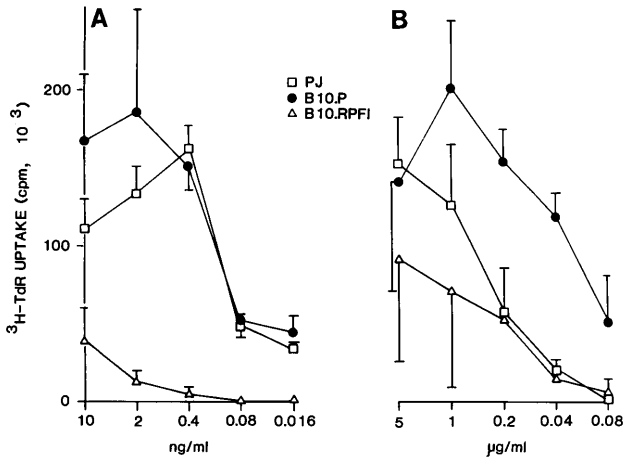

FIG. 5. Proliferative responses of lymphocytes from inbred PJ (E_α^+ *H-2*^{p}), congenic B10.P (E_α^+ *H-2*^{p}), and recombinant B10.RPF1 (E_α^- *H-2A*^{p}) mice to MAM (A) and SEB (B). See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

sponses of lymphocytes from mice heterozygous for the transgene (Fig. 6, DQ^+) with those from transgene-negative B10.M littermates (DQ^-) and from BALB/c, CBA, and C57BL/10 mice. Cells expressing the transgene gave high responses to MAM which were only slightly less than those obtained with BALB/c and CBA cells. Lymphocytes from transgene-negative B10.M littermates totally failed to respond to MAM. Interestingly, whereas cells from mice expressing only murine MHC molecules responded poorly to SEB, even at microgram levels, mice transgenic for DQ_βw6 gave very high responses to SEB (and SEA) (data not shown), even at 8 ng/ml. This observation supports the idea that the lower-level responses of murine cells to staphylococcal SAgs are exercised at the level of MHC presentation, at least for HLA-DQ molecules, rather than at the interaction with the TCR V_β chains on T cells and supports a role for the beta chain in SEA and SEB recognition.

A comparison of lymphocytes from mice expressing DQ_βw6 heterozygously (DQ^+) or homozygously (DQ^{++}) indicated that the latter exhibited proliferative responses to MAM that were equal to those observed for CBA mice which express E_α (Fig. 7). As before, responses to SEB were much higher in cells from DQ transgenic mice than in those from E_α-bearing mice (data not shown).

To determine the respective roles of A_α and DQ_β on MAM-induced responses, we examined the effect of antibodies to A_α^f and DQ_βw6 on the proliferative responses of B10.M mice transgenic for the DQ_βw6 chain (Table 4). Anti-DQ_β inhibited the responses to MAM by about 70%, whereas anti-A_α^f exhibited about 90% inhibition. Anti-E_α had no effect on MAM-induced proliferation in DQ_β-expressing mice at concentrations which totally inhibit the responses of E_α-bearing mice to MAM (data not shown), suggesting that different regions on E_α and DQ_β interact with MAM.

We next determined whether allelic polymorphism at the HLA-DQ locus influences lymphocyte responses to MAM, SEB, or SEA. We compared cells from mice doubly transgenic for both DQ_α and DQ_β chains to minimize the potential influence of altered configurations due to combination with the murine A_α chain in hybrid molecules. Mice expressing $DQ_{\alpha\beta}$w6 exhibited responses to MAM that were similar (Fig. 8) to those observed previously for mice exhibiting heterologous expression of DQ_βw6 in association with A_α^f (Fig. 6). In contrast, the responses to MAM of lymphocytes from mice expressing $DQ_{\alpha\beta}$w8 (Fig. 8) were markedly less than those from mice expressing $DQ_{\alpha\beta}$w6 and required approximately a

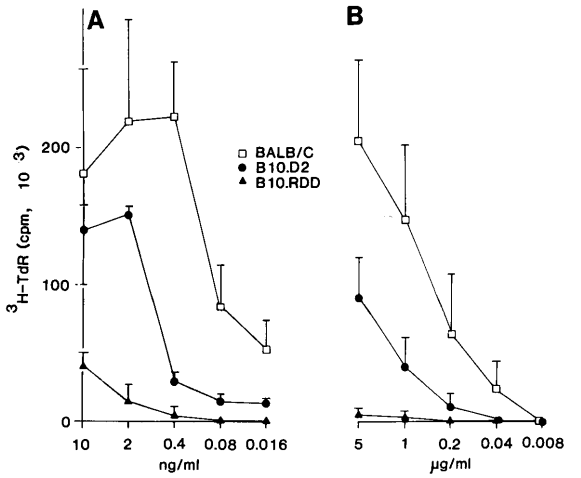

FIG. 4. Proliferative responses of lymphocytes from inbred BALB/c (E_α^+ *H-2*^{d}), congenic B10.D2 (E_α^+ *H-2*^{d}), and recombinant B10.RDD (E_α^- *H-2A*^{d}) mice to MAM (A) and SEB (B). See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

TABLE 3. Effects of antibodies to H-2A on lymphocyte responses to MAM

Antibody tested	Concn (μg/ml)	Specific uptake[a] of [^{3}H]TdR (cpm, 10^3) in response to MAM (0.4 μg/ml) with:	
		B10.RDD cells[b]	B10.BR cells[c]
None		27.6 ± 2.1	117.5 ± 2.4
Anti-H-2A^d	5	7.9 ± 1.9	128.3 ± 12.3
Anti-H-2A^k	4	26.7 ± 9.7	106.3 ± 25.3

[a] Specific uptake of [^{3}H]TdR is uptake in the presence of inducer minus uptake by cells alone.
[b] B10.RDD cells lack E_α and express H-2A^d.
[c] B10.BR cells express E_α and H-2A^k.

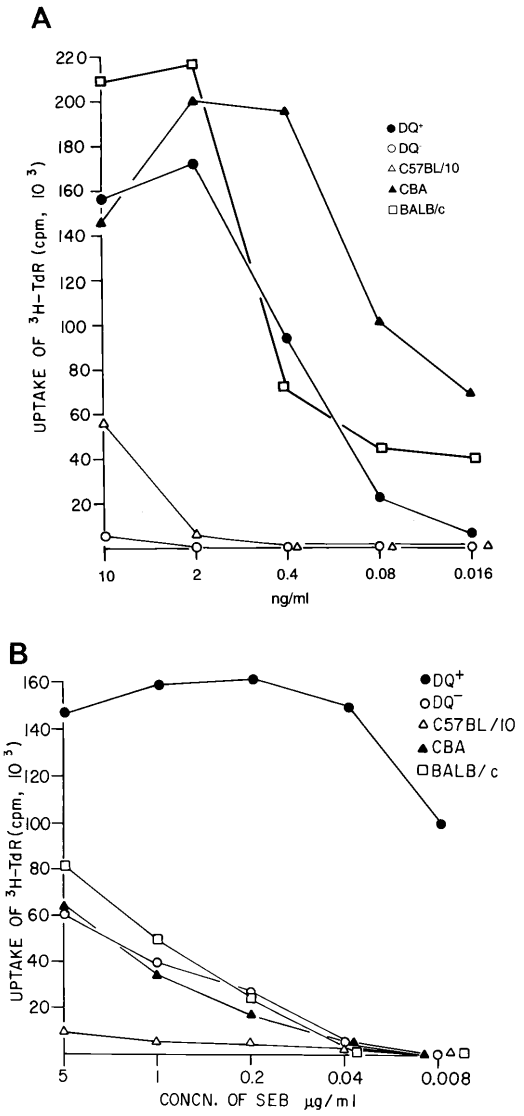

FIG. 6. Proliferative responses of inbred and transgenic mice to MAM (A) and SEB (B). Mice used were E_α^- transgenic mice of B10.M, *H-2*^{f} origin bearing the DQ_βw6 chain (DQ^+) or transgene-negative littermates (DQ^-). Controls were inbred C57BL/10 (*H-2*^{b}), CBA (*H-2*^{k}), and BALB/c (*H-2*^{d}) mice. Individual determinations for each mouse strain varied by less than 30%. See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

100-fold increase in the dose of MAM. In striking contrast, SEB and SEA both elicited higher responses from lymphocytes expressing $DQ_{\alpha\beta}$w8 (Fig. 8). The combined results indicate that allelic polymorphisms at the HLA-DQ locus also influence the reactivity of T cells to MAM and that human DQ_βw6 (the B1*0601 chain), in combination with A_α^f or DQ_αw6, presents MAM to T cells as effectively as does E_α. Furthermore, they confirm the strong preference of SEA and SEB for human HLA class II molecules.

DISCUSSION

The results presented here show that MAM can interact with a number of MHC class II molecules with and without the presence of E_α. First, we demonstrated that select H-2A molecules can present MAM and that polymorphisms at the locus encoding H-2A influence MAM reactivity. Second, by using transgenic mice, we showed that polymorphisms of HLA-DQ also influence reactivity to MAM and that lymphocytes expressing DQ_βw6 in combination with A_α^f or DQ_αw6 confer a very high responsiveness to MAM. Third, it has become clear that the pattern of reactivity to MAM for lymphocytes expressing various H-2A or HLA-DQ molecules was different from the pattern observed with SEB and SEA, suggesting that unique binding sites on either SAgs or class II molecules are used by these three SAgs.

By use of mice whose cells lacked H-2E expression due to the absence of the E_α gene, we demonstrated that lymphocytes from inbred and/or congenic and recombinant strains expressing the *b*, *d*, or *p* haplotypes at the locus encoding H-2A gave low but positive responses to MAM. Whereas lymphocytes from E_α^+ mice gave maximal responses at 2 to 0.4 ng/ml, responses from lymphocytes of E_α^- mice required at least a 100-fold increase in the concentration of MAM. In contrast, cells expressing the *k*, *q*, *f*, and *s* haplotypes were totally nonresponsive to MAM even when tested at concentrations of 100 ng/ml (unpublished data). A comparison of sequences of the H-$2A_\alpha$ chains present on MAM responder and nonresponder lymphocytes is shown in Table 5. Nonresponsiveness was associated with a typical pattern within the first domain of A_α. Namely, tyrosine was present at position 65 and glycine was present at position 66 in the first domain of A_α in mouse strains expressing the nonresponsive *k*, *q*, *f*, and *s* haplotypes.

We considered the possibility that the response of lymphocytes from some E_α^- mouse strains at high MAM concentrations might be due to a contaminant in the preparation. However, very recent work (38a) shows that homogeneous recombinant MAM has the identical dose response and pattern of reactivity for lymphocytes of different E_α^- inbred mouse strains. This pattern and dose response differs from that observed for lipopolysaccharide, a potential contaminant of recombinant proteins.

Another aim of this study was to investigate the interaction of MAM with HLA-DQ molecules. We found that the response of lymphocytes from E_α^- mice, transgenic for the $DQ_{\alpha\beta}$w8 genes, was low but comparable to that of H-2A-reactive mouse strains which fail to express a functional H-2E molecule. In striking contrast, the lymphocytes from mice expressing DQ_βw6 in association with A_α, or with DQ_αw6, exhibited proliferative responses to MAM that were comparable to those seen with cells expressing functional H-2E. It is not known whether these high responses are due to the DQ_β chain alone or whether DQ_β alters the conformation of A_α chains, thereby resulting in a higher avidity for MAM. However, lymphocytes from mice doubly transgenic for both the alpha and beta chains of DQw6, which preferentially form functional $DQ_{\alpha\beta}$ dimers, also give high responses to MAM. This might suggest that it is in fact the DQ_β chain that strongly interacts with MAM.

Interestingly, in vivo chimeric class II molecules consisting of DQ_βw6 in association with A_α^f or A_α^q behave like E_α-bearing class II molecules in deleting specific T cells expressing those Vβ TCR chains that associate with endogenous retroviral SAgs (60, 61). Surprisingly, doubly transgenic mice expressing $DQ_{\alpha\beta}$w6 did not delete the T cells, suggesting that some other factor, such as interaction with murine CD4, was also lost when both murine chains were replaced (43). This contrasts with MAM activation of T cells, which was very high in lymphocytes from doubly transgenic DQ-bearing mice.

In earlier work (3), transgenic animals expressing DR_α in place of E_α were found to present MAM well. Subsequently, Sawada and colleagues (47) used human B cells with a deletion

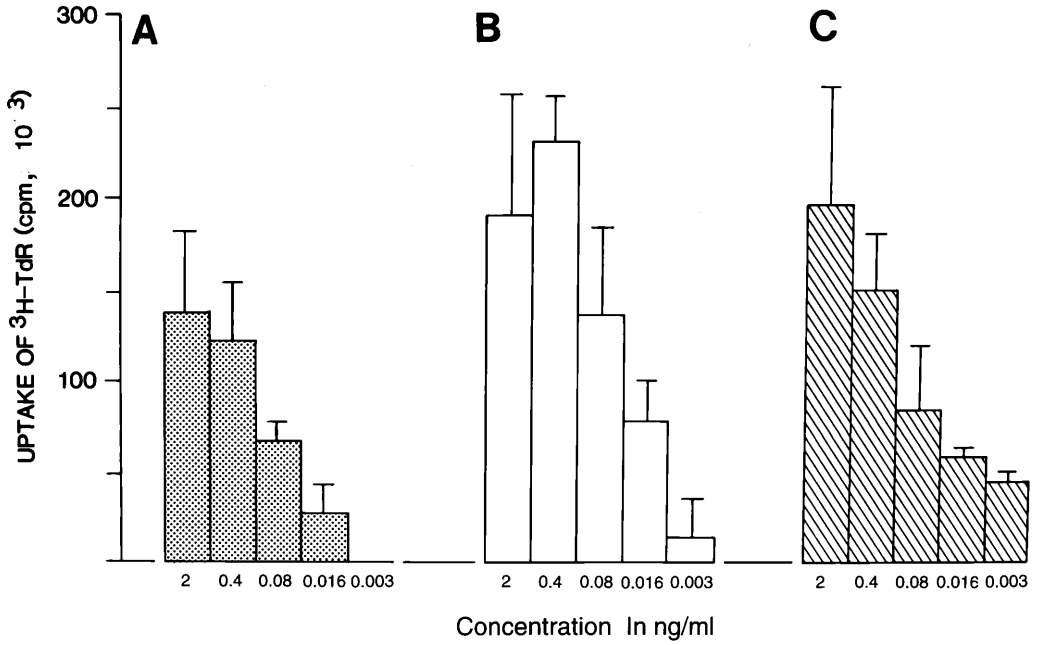

FIG. 7. Effect of heterozygous versus homozygous expression of DQ_βw6. B10.M mice carrying the human DQ_βw6 gene heterozygously (DQ^+) (A) or homozygously (DQ^{++}) (B) were compared with inbred E_α^+ CBA mice (C) for the ability of their lymphocytes to respond to MAM. See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

in DR_α as recipients of transfectant molecules comprising DR_α, E_α, or chimeric molecules in which the first α1 domain of E_α was replaced with the α1 domain of DR_α or in which the α1 domain of DR_α was replaced with the α1 domain of E_α. The results showed that E_α was somewhat more effective in presenting MAM than was DR_α and that the α1 domain of E_α had the largest influence on MAM presentation. The additional differences in presentation of MAM by MHC class II H-2A and HLA-DQ molecules found in the present study allowed us to hypothesize probable binding regions of the MHC class II molecule for MAM interaction. Comparison of the alpha chain sequences shows only four sites in the polymorphic first domain which could individually account for all of the responder and nonresponder types observed. These are amino acid residues at positions 31, 52, 65, and 66. Each of these has been mutated from the reactive E_α to the A_α form, and the resultant effect on peptide and MAM presentation has been tested (7). Each position is located along the α-helical edge of the peptide binding groove and, as expected, resulted in decreased peptide presentation. Surprisingly, none of the mutants had significant effects on MAM-induced proliferation. This suggests either that mutations at more than one site may be required or that the beta chain plays a larger role in determining MAM reactivity than was previously thought.

Major differences were noted in the pattern and degree of the lymphocyte responses observed with MAM and those observed with SEB and SEA (most data not shown). First, all strains that lacked E_α except one responded to the bacterial SAgs. Furthermore, the patterns of response of different *H-2A* haplotypes to MAM, SEB, and SEA were quite distinct. The influence of allelic polymorphisms at the H-2A locus on the presentation of staphylococcal SAgs to T cells has been noted previously (30, 31, 46, 53, 55, 59). Second, concentrations of SEB and SEA more than 1,000-fold higher than those of MAM were required to activate murine lymphocytes to similar levels. In contrast, lymphocytes from transgenic mice expressing different human HLA-DQ chains exhibited much higher responses to SEB and SEA than did mice expressing only murine MHC molecules. These data support the hypothesis proposed earlier by Fleischer and colleagues (23) that there is an evolutionary adaptation between a parasite, its SAg, and the manner in which it interacts with the immune system of its host. It is interesting, however, that although the proliferative response of human lymphocytes to MAM is less than the response observed with human lymphocytes, the doses required for 50% maximal stimulation are comparable for MAM, SEA, and SEB (16a).

Although MAM is not phylogenetically related to the bacterial or viral SAgs, evidence has been found for short regions of sequence similarity between these proteins (16, 38). Especially interesting is that residues 15 to 35 of the sequence of MAM peptide 2 (MAM_{15-35}), which blocks mitogenic activity, exhibits the most similarity to sequences of other SAgs (16). Of note is a cluster of residues at MAM_{29-36} which corresponds to residues 42 to 51 of the

TABLE 4. Effects of antibodies to DQ_βw6 and A_α^f on lymphocyte responses to MAM

Antibody treatment	Specific uptake[a] of [^{3}H]TdR (cpm, 10^3) by lymphocytes[b] in response to MAM at 0.08 ng/ml	
	Expt 1	Expt 2
None	72.5 ± 8.8	52.7 ± 4.2
Anti-DQ_β (100 μg/ml)	25.6 ± 2.3	16.1 ± 0
Anti-A_α^f (55 μg/ml)	7.0 ± 1.9	5.9 ± 1.6
Anti-E_α (1:20 dilution)	56.9 ± 2.9	41.4 ± 0.7

[a] Specific uptake of [^{3}H]TdR is uptake in the presence of inducer minus uptake by cells alone.

[b] Splenocytes were from B10.M mice carrying the DQ_βw6 transgene (HLA-DQB1*0601).

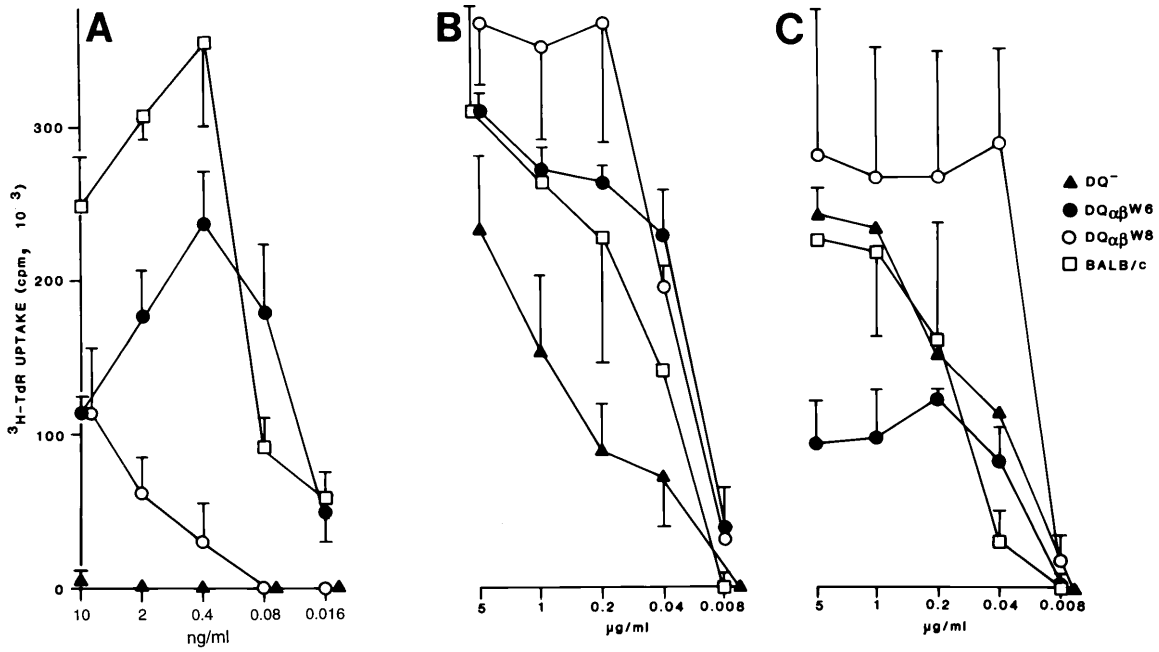

FIG. 8. Role of allelic polymorphisms at HLA-DQ on proliferative responses to MAM (A), SEA (B), and SEB (C). The mice used were E_α^- B10.M mice with no transgene (DQ^-), B10.M background mice expressing the $DQ_{\alpha\beta}$w6 gene, and mice expressing $DQ_{\alpha\beta}$w8. E_α^+ BALB/c mice were used as controls. See Fig. 1 legend for explanation of expression of data and haplotype of mice used.

streptococcal pyrogenic exotoxin A (SPEA) region, some of which ($SPEA_{42}$ and $SPEA_{45}$) are important for binding with HLA-DQ molecules (37). MAM_{15-35} also contains a group of residues similar to those known to be important for SEB binding to MHC molecules (36). Although SPEA binds preferentially to HLA-DQ (34, 43), SEA binds only to HLA-DR (22) and toxic shock syndrome toxin 1 and SEB have been reported to bind to both molecules (51). Evidence of cross-reactivity between the unrelated SEA and mouse mammary tumor virus 7 SAgs has also been presented by Torres and colleagues (56).

The precise reasons for the high reactivity to MAM of lymphocytes bearing the E_α or DQ_β*0601 chains remains to be determined. In addition, candidate active residues on these molecules as well as on A_α need to be verified by mutational studies. Further work on the three-dimensional structure of MAM and its association with class II MHC molecules ultimately will define the molecular basis of these interactions as it has already done for several other bacterial SAgs. Largely on the basis of such crystallographic (35, 49, 54) and mutational studies (33, 36), it is now known that SEA binds two distinct sites on class II molecules. One, a high-affinity site on the class II beta chain, requires Zn^{2+} for optimal binding, whereas the other is a low-affinity site on the alpha chain (33). For SEB, only the lower-affinity alpha chain site has been shown. Evidence has been presented by Bernatchez et al. (5) and ourselves (reference 48 and present study) to suggest that at least two sites are recognized by MAM as well, including both alpha and beta chains. Each may be similar to those of SEA, including a dependency on Zn^{2+} for maximal binding as was observed for SEA (5).

SAgs have been hypothesized to play a role in autoimmune disease based upon their ability to clonally expand autoreactive T cells and to induce polyclonal B-cell activation with production of autoantibodies (25, 29, 32, 45, 48, 50). MAM activates the same lymphocytes that drive the autoreactive response in mice with experimental collagen-induced arthritis. Notably, MAM has been shown to cause flares in disease activity and can trigger arthritis in animals suboptimally immunized with type II collagen (15). Furthermore, MAM is a polyclonal B-cell activator of both murine (57) and human lymphocytes (17) and can also lead to increased production of rheumatoid factor in lymphocytes taken from patients with rheumatoid arthritis and from normal individuals (21). Characteristically, autoimmune diseases are associated with host possession of specific class II molecules. For example, rheumatoid arthritis is frequently associated with expression of select DR4 and DR1 haplotypes. Thus, the differential ability of SAgs to activate lymphocytes expressing specific class II MHC alleles provides one mechanism whereby these proteins might contribute to autoimmune disease susceptibility.

TABLE 5. Association of responses to MAM with MHC sequence

MHC molecule	MAM response[e]	Sequence[a] region within the first domain
		65[b] 66[b]
		↓ ↓
A_α^k	−	N I A T G K H N L E I L T K
A_α^q	−	.[c] . . . . . G G W . .
A_α^f	−	E . . . . . G . . . .
A_α^s	−	. . . . . Y T . G . . . .
A_α^d	+	. . . A E . . . . G . . . .
A_α^b	+	. . . V V . . . . G V . . .
E_α	+++	. . . V D . A N . D V M K E
		↓ ↓
		(T G)[d]

[a] Sequences were reported by Davis et al. (18) and McNicholas et al. (41).
[b] Residues important for peptide presentation at these positions are indicated.
[c] Period denotes identity with the A_α^k sequence.
[d] Mutations made in E_α (6) are shown in parentheses.
[e] Lymphocyte proliferation: −, negative; +, low; +++, high.

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