

Influence of Major Histocompatibility Complex Haplotype on the Mitogenic Response of T Cells to Staphylococcal Enterotoxin B

JOHN H. ROBINSON,^{1*} GWYN PYLE,^{1,2} AND MICHAEL A. KEHOE²

Departments of Immunology¹ and Microbiology,² The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom

Received 6 May 1991/Accepted 25 July 1991

The abilities of antigen-presenting cells (APC) from nine independent major histocompatibility complex haplotypes and a number of intra-H-2 recombinant congenic strains of mice to present staphylococcal enterotoxin B (SEB) and induce proliferation in murine T-cell receptor V β 8⁺ T-cell clones were compared. SEB presented by APC of all haplotypes tested induced significant responses in each of the T-cell clones. The magnitude of response was similar for most haplotypes, but there were limited quantitative differences between certain haplotypes. SEB presented by APC from H-2^b mice as well as the intra-H-2 recombinant strains B10.GD and B10.A(4R), which do not express cell surface I-E (designated I-E⁻), induced the poorest T-cell responses. However, APC from A^fE⁻, A^eE⁻, and A^gE⁻ mice were as potent in SEB presentation as APC expressing both I-A and I-E. Antibodies against I-E were more effective than anti-I-A antibodies at inhibiting responses to SEB presented by APC expressing both I-A and I-E, whereas responses induced by APC expressing I-A but not I-E were blocked by antibodies against I-A. Thus, our results show that I-A can present SEB efficiently but that expression of both I-A and I-E on the same APC results in presentation of SEB predominantly by I-E. In addition, experiments using four distinct I-E⁻ strains of mice indicate that I-A alleles differ in their ability to present SEB.

Staphylococcus aureus enterotoxins (SEA, SEB, SEC1, SEC2, SEC3, SEE, and SED) have been recognized for many decades as one of the major causes of food poisoning (for a review, see reference 1) and more recently as potential virulence factors in some cases of toxic shock syndrome (2, 4, 5, 17) and septicemia (10). The SEs belong to a family of potent T-cell mitogens called superantigens (for reviews, see references 12 and 15), and it has been suggested that cytokine production resulting from their mitogenic activity may play an important role in pathogenesis (14, 22). Their mitogenic activity is strictly dependent on antigen-presenting cells (APC) expressing cell surface major histocompatibility complex (MHC) class II molecules (7, 12, 22), but their mode of action is distinct from the mechanism of conventional antigen presentation. SEs bypass the normal specificity of the T-cell receptor (TCR) for both a specific T-cell epitope and self-MHC class II, probably by cross-linking MHC class II and TCR molecules via sites that are distinct from those involved in presentation of conventional antigens (6). SEs require an intact disulfide bridge for mitogenicity (8), can be presented by non-self- or even xenogenic MHC class II (reviewed in reference 12), and, unlike many polyclonal mitogens, selectively activate T cells bearing particular TCR V β segments (3, 13, 22). However, since each V β family includes TCRs with many different epitope specificities, superantigens can induce the nonspecific proliferation of a substantial proportion of the total T-cell population.

MHC class II molecules of both I-A and I-E loci are expressed on APC of the majority of inbred strains of mice. However H-2^b, H-2^f, H-2^s, and H-2^g haplotypes do not express cell surface I-E molecules because of either gene defects or abnormal mRNA processing (16). A number of studies have suggested that mouse T-cell responses to SEs are influenced by the APC MHC haplotype, and it has been

shown that alleles and isotypes of human MHC class II molecules differ in their ability to present SEs to mouse T-cell hybridomas (9). Significant quantitative differences have been observed between the SEB-induced responses in spleen cells from different H-2 haplotypes, and inhibition experiments with monoclonal antibodies against I-A or I-E have indicated that SEB is presented to T cells predominantly by I-E molecules (23). However, spleen cell responses do not distinguish between effects due solely to the APC MHC class II haplotype and effects due to other potential differences between strains, such as differences in the proportions of SEB-responsive TCR V β families in polyclonal T-cell populations. Few reports describe the presentation requirements of SEB to T-cell clones, and these compare only a limited number of murine MHC class II haplotypes (21, 23).

In order to examine the influence of the MHC class II locus and haplotype on T-cell responses to SEB, we have examined the responses of T-cell clones to SEB presented by APC from inbred mice of nine independent H-2 haplotypes. Here we report that each of the T-cell clones responds to SEB presented by APC from all MHC haplotypes tested and that the magnitude of response is similar for most haplotypes. In addition, we confirm that SEB is preferentially presented by I-E molecules when the APC expresses both I-A and I-E molecules but show that some but not all I-A alleles can present SEB efficiently in the absence of I-E. Thus, there is no simple correlation between the ability to express I-E on the APC surface and effective presentation of SEB.

MATERIALS AND METHODS

Mouse strains. The characteristics and sources of inbred mouse strains used are described in Table 1.

SEB. SEB was purchased from Sigma Chemical Co. (Poole, Dorset, United Kingdom), and purity was confirmed

* Corresponding author.

TABLE 1. Haplotypes and source of inbred mice

Strain	Allele ^a							Source ^b
	H-2	K	A α β	E α β	D	Mls1	Mls2	
BALB.B	b	b	b b	b b	b	b	b	1
BALB/c	d	d	dd	dd	d	b	a	2
BALB.K	k	k	kk	kk	k	b	a	1
B10	b	b	b b	b b	b	b	b	1
B10.A(2R)	h2	k	kk	kk	b	b	b	1
B10.A(4R)	h4	k	kk	b k	b	b	b	1
B10.A(5R)	i2	b	bb	kb	d	b	b	1
B10.BR	k	k	kk	kk	k	b	b	1
B10.D2	d	d	dd	dd	d	b	b	1
B10.GD	g2	d	dd	b d/b	b	b	b	2
B10.M	f	f	ff	ff	f	b	b	1
B10.RIII	r	r	rr	rr	r	b	b	1
B10.S	s	s	ss	ss	s	b	b	1
CBA/Ca	k	k	kk	kk	k	b	b	3
CBA/J	k	k	kk	kk	k	a	b	1
C3H-NB	p	p	pp	pp	p	b	a	1
C57BL/6	b	b	bb	bb	b	b	b	1
DBA/1	q	q	qq	qq	q	a	a	1
DBA/2	d	d	dd	dd	d	a	a	1
NZW	z	z	zz	zz	z	b	a	1
SJL	s	s	ss	ss	s	b	a	1
SWR	q	q	qq	qq	q	a	b	4
129	b	b	bb	bb	b	b	a	5

^a Alleles in boldface type are not expressed.

^b 1, Olac 1976 Ltd, Cirencester, United Kingdom; 2, National Institute for Medical Research, London, United Kingdom; 3, Bantin and Kingman Ltd., Hull, United Kingdom; 4, Imperial Cancer Research Fund Laboratories, London, United Kingdom; 5, Clinical Research Centre, Harrow, United Kingdom.

by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Batches which contained visible contaminating bands on Coomassie blue-stained gels were further purified by preparative isoelectric focusing in a horizontal Rotaphore chamber (Bio-Rad Laboratories Ltd., Hemel Hempstead, Hertfordshire, United Kingdom) to >95% purity as judged by SDS-PAGE. Purity was confirmed by the observation that SEB-activated BALB/c spleen cells comprised a total of >90% V β 8-, V β 7-, and V β 3-bearing T-cell blasts with no increase in V β 11, as assessed by immunofluorescence using F23.1 (anti-V β 8) and KJ25 (anti-V β 3) antibodies (provided by C. G. Brooks, Department of Immunology, University of Newcastle upon Tyne) as well as fluorescein isothiocyanate-conjugated monoclonal rat anti-mouse V β 7 and V β 11 antibodies (Pharmingen; A.M.S. Biotechnology Ltd., Burford, Oxford, United Kingdom).

T-cell clones. Clones S7 and R28 were cloned from BALB/c mice previously immunized with recombinant group A streptococcal serotype 5 M protein and are specific for two distinct M5 epitopes in the context of A^d (19). Clones 6D and N13 were cloned from C57BL/6 mice and are specific for ovalbumin in the context of A^b (20). Clones N13, S7, and R28 were shown to be V β 8.3⁺ and clone 6D was shown to be V β 8.1⁺ by indirect immunofluorescence using F23.1 (anti-V β 8), F23.2 (anti-V β 8.2), and KJ16 (anti-V β 8.1 and V β 8.2) antibodies (provided by C. G. Brooks).

Proliferation assays. Proliferation responses were assayed in triplicate cultures in 200 μ l of RPMI 1640 medium (GIBCO Ltd., Paisley, Scotland) supplemented with 3.0 mM L-glutamine (GIBCO Ltd.) and 10% (vol/vol) fetal bovine serum (Sigma Chemical Co.). Cloned T cells (2×10^4) were incubated for 48 h at 37°C in 5% CO₂ with 7.5×10^5 irradiated (20 Gy) spleen cells (APC) in a microtiter plate

with flat-bottomed wells. Cells were pulse-labelled for the final 4 h of culture with 0.4 μ Ci of ³H-thymidine (specific activity, 2 Ci/mM; Amersham International plc., Amersham, Buckinghamshire, United Kingdom) per well, and then radioactivity was quantitated by scintillation spectroscopy. Results are expressed as mean disintegrations per minute of triplicate cultures.

Monoclonal antibodies. Monoclonal antibodies against I-A and I-E were prepared by ammonium sulfate precipitation of culture supernatants from the following antibody-secreting hybridomas: H81-208-22.6 (immunoglobulin G2a [IgG2a], anti-E, anti-Ia-7; provided by E. L. Simpson, Clinical Research Centre, Harrow, Middlesex, United Kingdom); Y-3P (IgG2a, anti-A α ^b, -A α ^f, -A α ^p, -A α ^q, -A α ^r, -A α ^s, -A α ^u, and -A α ^v), Y-17 (IgG2a, anti-E α ^k, -E α ^b, -E α ^r, -E α ^s, and -E α ^v), 14-4-4S (IgG2a, anti-E α ^d, -E α ^k, -E α ^p, and -E α ^r), 26.8.16S (IgM, anti-A^k) and 25-9-3S (IgM, anti-A^b), obtained from the American Type Culture Collection, Rockville, Md: and m122 (IgG, anti-A^d) and m126 (IgG, anti-A^d) (provided by D. Klein, Max Planck Institute for Biology, Tübingen, Germany).

Terminology. The term E⁻ is used to denote APC that fail to express correctly assembled I-E molecules on their cell surface. Mice of H-2^f and H-2^g haplotypes are unable to express any I-E molecules because of defects in synthesis of both the E α and E β chains (16). H-2^b and H-2^s haplotypes have deletions in the E α gene and fail to express I-E molecules on the cell surface. However, E β chains which could associate with A α chains to be expressed at low density, as has been shown for A β ^dE α in transfection experiments (18), are synthesized.

RESULTS

Effect of MHC haplotype. APC from inbred mice of H-2 haplotypes b, d, f, k, p, q, r, s, and z were examined for their ability to present SEB to T-cell clones bearing TCR of the SEB-responsive V β 8 family. Control experiments omitting SEB showed that the T-cell clones failed to respond to spleen cells from the Mls1^a strain SWR, indicating that none of the four clones were Mls1^a responsive. Similarly, in controls omitting the T-cell clones, no responses to SEB were observed (10^{-6} to 10^{-9} M), ruling out a contribution to responses by T cells among the irradiated spleen APC (data not shown). The proliferative responses of each T-cell clone to a range of SEB concentrations presented by APC from either eight (clones 6D and N13) or nine (clones S7 and R28) H-2 haplotypes were compared (Fig. 1). APC from all MHC haplotypes, including those which do not express I-E molecules on the APC surface (H-2^b, H-2^f, H-2^g, and H-2^s), presented SEB and induced significant proliferative responses in all four T-cell clones. SEB presented by APC of most haplotypes, irrespective of I-E expression, induced similar responses in the T-cell clones. The relative magnitude of response of each clone to SEB presented by APC from different MHC haplotypes was consistent between experiments. There are, however, limited quantitative differences in T-cell clone responses to SEB presented by certain APC haplotypes; e.g., SEB presented by APC from H-2^b mice induced the poorest responses. Other haplotypes induce poorer responses in only a proportion of the clones, e.g., H-2^g and H-2^z in clone S7, which has a greater affinity for SEB than other clones, as shown by the 100-fold-lower doses of SEB used.

In addition, SEB presentation by APC from MHC-compatible mice with different genetic backgrounds were also

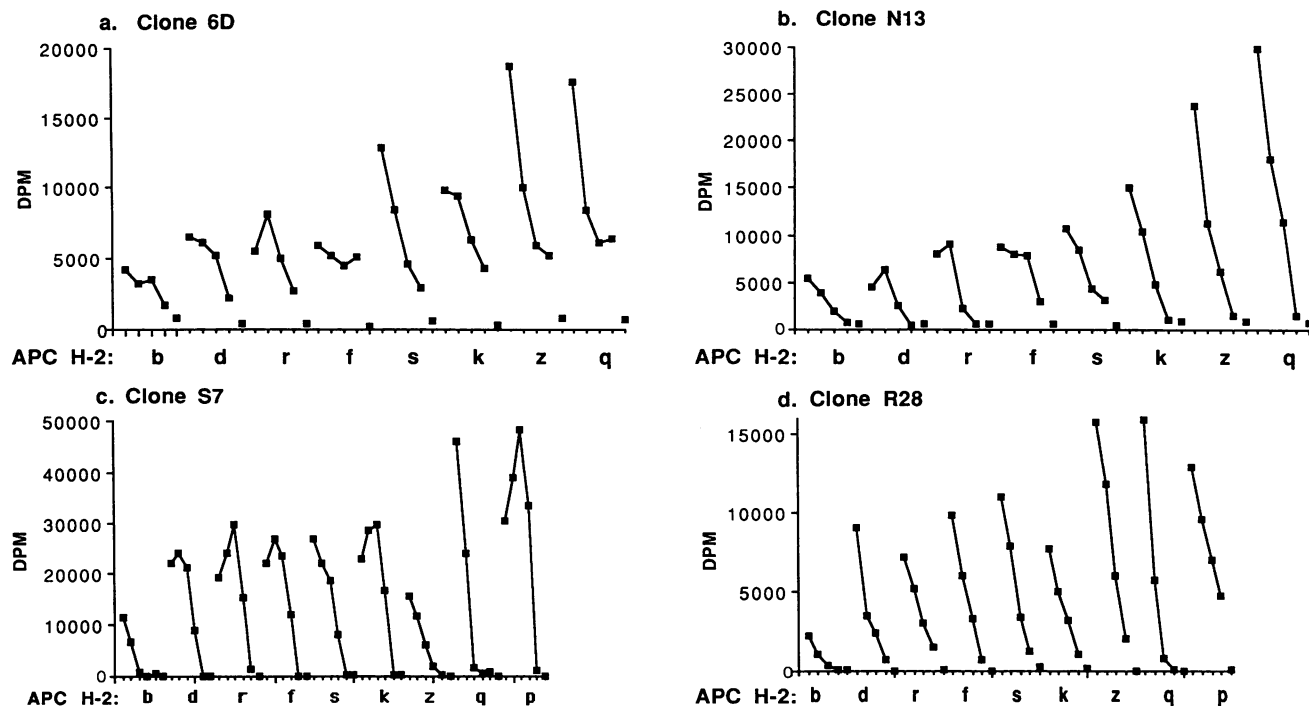


FIG. 1. Proliferation responses of T-cell clones to SEB presented by APC from mice of different MHC haplotypes. (a and b) Proliferation responses of C57BL/6 clones 6D and N13 to SEB presented by APC from H-2^b (C57BL/6), H-2^d (BALB/c), H-2^f (B10.RIII), H-2^f (B10.M), H-2^s (SJL), H-2^k (CBA/Ca), H-2^z (NZW), and H-2^q (SWR) mice. (c and d) Proliferation responses of BALB/c clones S7 and R28 to SEB presented by APC from H-2^b (B10), H-2^d (B10.D2), H-2^f (B10.RIII), H-2^f (B10.M), H-2^s (SJL), H-2^k (B10.BR), H-2^z (NZW), H-2^q (SWR), and H-2^p (C3H-NB) mice. On each curve, the points from left to right correspond to the following concentrations of SEB: 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹, and 0 M for 6D, N13, and R28 and 10⁻⁷, 10⁻⁸, 10⁻⁹, 10⁻¹⁰, 10⁻¹¹, and 0 M for clone S7.

compared. There were no clear differences in the T-cell responses to SEB presented by APC from different strains of H-2^b (strains BALB.B, B10, C57BL/6, and 129), H-2^k (strains BALB.K, CBA/J, CBA/Ca, C3H/He, and B10.BR), H-2^s (B10.S and SJL), H-2^q (SWR and DBA/1), or H-2^d (B10.D2, BALB/c, and DBA/2) mice (data not shown), suggesting a minimal influence of non-MHC genes on SEB responses in the strain combinations used.

Effect of I-A and I-E loci. In the experiments described in the legend to Fig. 1, SEB presented by APC from H-2^b mice, which fail to express I-E, induced poor responses to SEB in T-cell clones. These results are consistent with previous reports that SEB is presented predominantly by I-E molecules, based partly on anti-class II antibody inhibition experiments (23) and partly on the induction of poor responses by SEB in spleen cells from B10.GD and B10.A(4R) mice, which fail to express I-E (11, 14). However, Fig. 1 shows that SEB presented by APC from H-2^f, H-2^s, and H-2^q mice, which also lack I-E, induced responses in T-cell clones that are generally equivalent to I-E-expressing haplotypes, indicating that I-A alleles differ in their ability to present SEB. To further investigate the contribution of I-E and I-A molecules to SEB presentation, anti-class II antibody blocking experiments were performed. The ability of antibodies against MHC class II to inhibit T-cell clone responses to SEB presented by H-2^b, H-2^d, and H-2^k APC were examined (Fig. 2). Antibodies against I-E blocked proliferation effectively when SEB was presented by H-2^d and H-2^k APC but not by H-2^b APC. The response to SEB presented by H-2^b APC was blocked by anti-I-A antibodies (Fig. 2b), whereas the responses of the same T-cell clones to SEB presented by H-2^d or H-2^k APC were not (Fig. 2a and c), although partial

blocking with anti-I-A was observed in some experiments using B10.BR and B10.D2 APC (data not shown). The failure of antibody Y-17, which reacts with E α ^b, to block presentation by H-2^b APC argues against presentation of SEB by hybrid A α E β molecules, which have been shown to be expressed on the surface of transfected L cells (18). In addition, Y3P antibody blocked proliferation induced by SEB presented by B10.S or DBA/1 APC (data not shown), directly demonstrating that SEB is presented effectively by I-A in other I-E⁻ strains.

Consistent with previous reports, the T-cell clones used in this study respond poorly to SEB presented by APC from B10.A(4R) (A^kE⁻) and B10.GD (A^dE⁻) mice compared with those from B10.A(2R) (A^kE^k) and B10.D2 (A^dE^d) mice (Fig. 3). Also, SEB is presented more effectively by APC from B10.A(5R) mice (A^bE α ^k β ^b) than by APC from C57BL/6 mice (A^bE⁻) (Fig. 3). However, presentation of SEB by C57BL/6 was more efficient than that by B10.A(4R) or B10.GD. The responses of polyclonal splenic T cells from these strains are consistent with the T-cell clone responses (data not shown). Finally, presentation of SEB to T-cell clones by B10.A(4R) and B10.GD APC was blocked effectively by antibodies against I-A (Fig. 4a and b), whereas responses induced by B10.A(5R) APC were blocked by antibodies against both I-E and I-A, with more profound inhibition by anti-I-E. Blocking by 14-4-4S against E α also indicated that the E α ^k β ^b molecule can present SEB. Neither the antibody inhibition experiments nor the comparison of intra-H-2 recombinant mice revealed any major discrepancies with data reported by others (11, 14, 21, 23). However, taken together, our results show that I-A^b, I-A^d, and I-A^k can present SEB but that

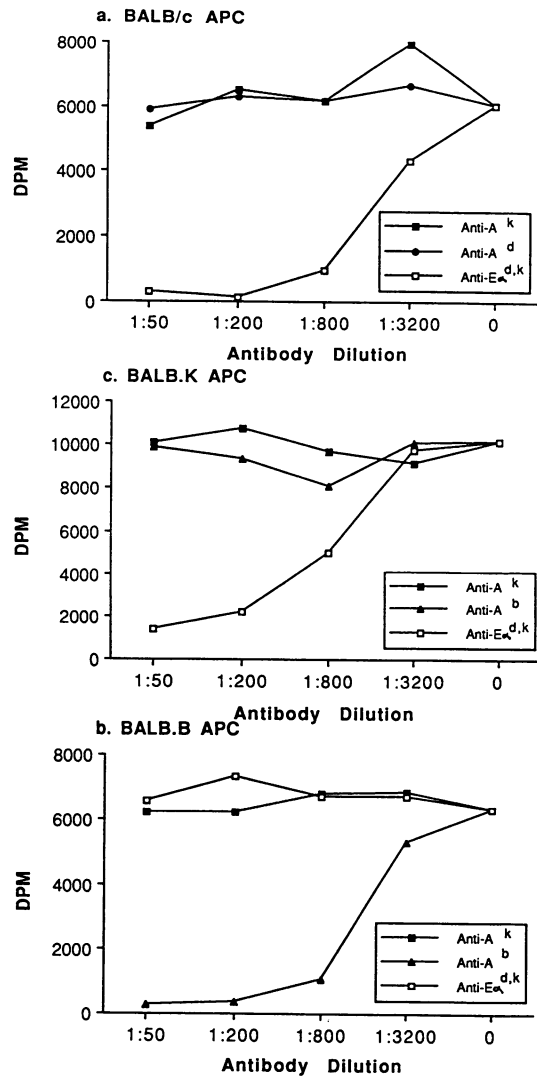


FIG. 2. Inhibition of T-cell clone proliferation to SEB by anti-MHC class II antibodies. The responses of clone R28 to SEB presented by BALB/c (H-2^d), BALB.B (H-2^b), and BALB.K (H-2^k) splenic APC are shown. The SEB concentrations used, 10^{-8} M (a), 10^{-7} M (b), and 10^{-8} M (c), were those which produced 50% of the maximum response for the particular clone when presented by the relevant APC haplotype in preliminary dose-response experiments in the absence of antibody. The experiment used antibodies 26-8-16S (A^k), Y3P (A^b), m122 (A^d), and H81-208-22.6 (E^d and E^k). Similar blocking was seen with 14-4-4S (E^d and E^k) in panels a and c, Y-17 (E^k) in panel c, and 25-9-3S (A^b) in panel b. Clone N13 gave essentially the same results.

expression of both I-A and I-E on the same APC results in presentation of SEB predominantly by I-E.

DISCUSSION

Several previous studies indicated that there are significant quantitative differences in SEB-induced polyclonal T-cell responses between different MHC haplotypes of mice (11, 14, 23). It has been demonstrated that polyclonal splenic T cells from B10.GD or B10.A(4R) mice, which do not express I-E, responded poorly or not at all to SEB (11, 14). Furthermore, antibodies against I-E were shown to inhibit a

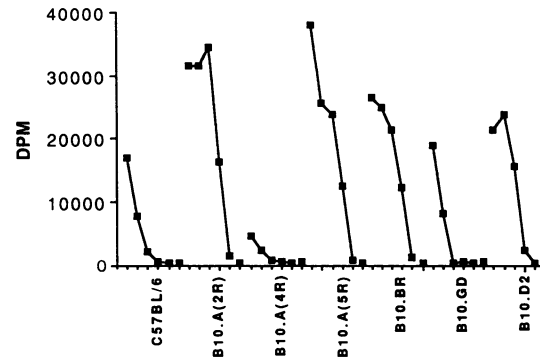


FIG. 3. T-cell clone R28 proliferation response to SEB presented by APC from intra-H-2 recombinant, congenic B10 mice (Table 1). Results from B10.GD and B10.D2 were taken from a separate experiment. Clone S7 gave essentially the same results. The points from left to right correspond to SEB doses of 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , and 0 M.

much larger proportion of the SEB-induced responses of spleen cells from A^kE^k or A^dE^d mice than antibodies against I-A. In addition, Yagi et al. (23) reported that SEB-induced mitogenic responses of 13 T-cell clones were absolutely I-E restricted, suggesting that individual T cells responded to SEB presented by either I-A or I-E, but not both, and that the majority of clones respond to SEB presented by I-E. Taken together, these studies suggested that although there is no absolute requirement for I-E, SEB is presented to T cells predominantly by I-E molecules. While the results described in this paper are consistent with those of most previous experiments (11, 14, 21, 23), they point to different conclusions concerning the influence of the APC MHC class II haplotype on mouse T-cell responses to SEB. Previous conclusions were based on comparisons among a limited number of mouse strains and inhibition by anti-MHC class II antibodies. In this study, the responses of T-cell clones to SEB presented by APC from a wide range of mouse strains were compared. While individual T-cell clones varied to some extent in their responses to SEB, APC from inbred mice of nine independent MHC haplotypes presented SEB effectively, inducing significant proliferation in a number of SEB-responsive T-cell clones. Similar results were obtained when the SEB-induced responses of spleen cells from these mice were compared (data not shown). Therefore, the APC MHC class II haplotype had only a limited effect on these responses. Although the limited differences between certain haplotypes might reflect differences in the affinity of SEB for the polymorphic regions of MHC class II molecules, other differences between APC, such as MIs disparity, could also influence the response in some haplotypes. However, three of the clones used were V β 8.3⁺, which is not a known MIs-responding phenotype. The fourth clone was typed as V β 8.1⁺, which is an MIs1^a-responding subfamily of TCR but was shown not to respond to SWR spleen cells which express MIs1^a (Fig. 1).

Consistent with previous studies (11, 14), we have shown that SEB presented by APC from B10.A(4R) and B10.GD mice induced poor responses. In addition, antibodies against I-E were more effective than anti-I-A antibodies in inhibiting T-cell clone responses to SEB presented by APC expressing both I-A and I-E. However, responses were blocked by antibodies against I-A if APC which express I-A but not I-E were used, suggesting that I-A can present SEB effectively

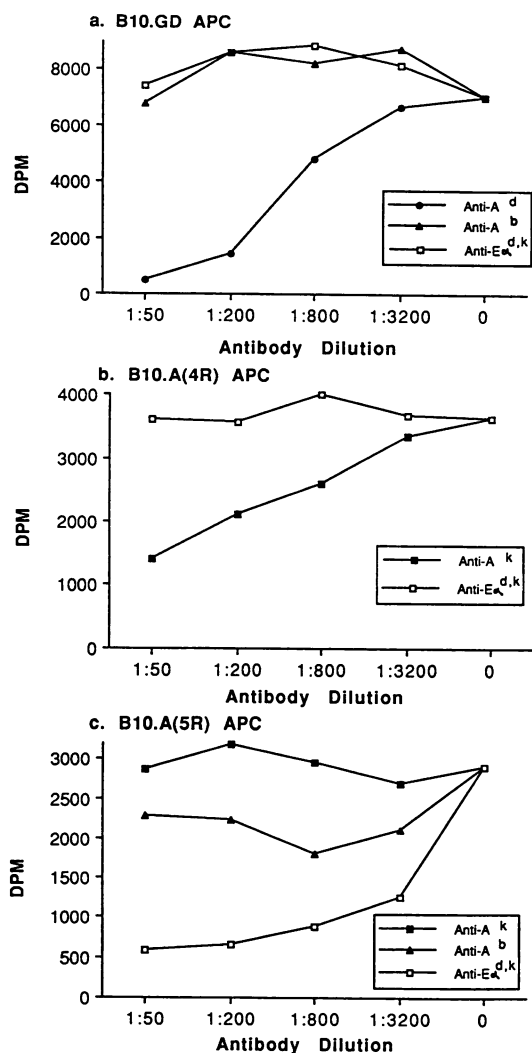


FIG. 4. Inhibition of T-cell clone proliferation to SEB by anti-MHC class II antibodies. The responses of clone R28 to SEB presented by B10.GD (A^{dE^-}), B10.A(4R) (A^{kE^-}), and B10.A(5R) (A^{bE^-}) splenic APC are shown. The SEB concentrations used were 10^{-7} M (a) 10^{-7} M (b), and 10^{-8} M (c). The experiment used antibodies 26-8-16S (A^k), Y3P (A^b), m122 (A^d), and 14-4-4S (E^d and E^k).

in the absence of I-E. In addition, the demonstration that all four T-cell clones could respond to SEB presented by APC which lack I-E and were profoundly blocked by antibodies against I-E in the presence of I-E-expressing APC shows that these clones can recognize SEB presented by both I-A and I-E molecules. This contrasts with the conclusion of Yagi et al. (23), which suggested that individual T cells can recognize SEB presented by either I-A or I-E but not both. However, consistent with our findings, White et al. (22) have reported that two of three T-cell hybridomas tested responded to SEB presented by B lymphoma cells expressing either A^d alone or E^d alone. Although SEB recognition by individual T cells might be absolutely I-E restricted in some cases, both our data and those of White et al. (22) suggest that this is the exception rather than the norm.

The finding that both spleen cells and T-cell clones responded well to SEB presented by H-2^f, H-2^s, or H-2^a APC

which lack surface I-E molecules was surprising in view of previous studies. The demonstration that among I-E-nonexpressing strains of mice, C57BL/6, B10.A(4R), and B10.GD APC present SEB poorly whereas B10.M, B10.S, and SWR present SEB well suggests that there is heterogeneity in the ability of different alleles of I-A to present SEB. This could be explained by differences in the relative affinities of I-A alleles for SEB. Our results would be consistent with A^d , A^k , and, to a lesser extent, A^b molecules having lower affinities than A^f , A^s , and A^a . In the case of H-2^k and H-2^d APC, the low affinity of A^d and A^k for SEB appears to be compensated by the expression of I-E molecules, whereas the low affinity of A^b for SEB cannot be compensated in H-2^b APC. This might explain why lower T-cell responses are consistently observed with this haplotype. The experiments described here would not identify differences in the affinity of SEB for different alleles of I-E molecules, but the relatively uniform responses induced in T-cell clones by the five I-E expressing haplotypes of APC used suggests that in contrast to I-A, SEB binding to I-E is not influenced by haplotype. It would be interesting to examine this further by using a series of I-E-transfected fibroblasts and testing their ability to present SEB to the same panel of T-cell clones.

ACKNOWLEDGMENTS

This work was supported by project grant 2043/92 from The UK MOD.

We thank E. L. Simpson, Clinical Research Centre, Harrow, Middlesex, United Kingdom, for the gift of the H81-208-22.6 hybridoma; D. Klein, Max Planck Institute for Biology, Tubingen, Germany, for the anti-m122-secreting hybridoma; and C. G. Brooks, Department of Immunology, University of Newcastle upon Tyne, for F23.1, F23.2, KJ16, and KJ25 antibodies.

REFERENCES

- Bergdoll, M. S. 1983. Enterotoxins, p. 559-598. In C. S. F. Easman and C. Adlam (ed.), *Staphylococci and staphylococcal infections*, vol. 2. Academic Press, Inc., London.
- Bohach, G. A., B. N. Kreiswirth, R. P. Novick, and P. M. Schlievert. 1989. Analysis of toxic shock syndrome isolates producing staphylococcal enterotoxins B and C1 with use of Southern hybridization and immunologic assays. *Rev. Infect. Dis.* 11(Suppl. 1):S75-S81.
- Choi, Y., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler. 1989. Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. *Proc. Natl. Acad. Sci. USA* 86:8941-8945.
- Crass, B. A., and M. S. Bergdoll. 1986. Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. *J. Clin. Microbiol.* 23:1138-1139.
- Crass, B. A., and M. S. Bergdoll. 1986. Toxin involvement in toxic shock syndrome. *J. Infect. Dis.* 153:918-926.
- Dellabona, P., J. Peccoud, J. Kappler, P. Marrack, C. Benoist, and D. Mathis. 1990. Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell* 62:1115-1121.
- Fleischer, B., and H. Schrezenmeier. 1988. T cell stimulation by staphylococcal enterotoxins. Clonally variable response and requirements for major histocompatibility complex class II molecules on accessory or target cells. *J. Exp. Med.* 167:1697-1707.
- Grossman, D., R. G. Cook, J. T. Sparrow, J. A. Mollick, and R. R. Rich. 1990. Dissociation of the stimulatory activities of staphylococcal enterotoxins for T cells and monocytes. *J. Exp. Med.* 172:1831-1841.
- Herman, A., G. Croteau, R.-P. Sekaly, J. Kappler, and P. Marrack. 1990. HLA-DR alleles differ in their ability to present staphylococcal enterotoxins to T cells. *J. Exp. Med.* 172:709-717.
- Humphreys, H., C. T. Keane, R. Hone, H. Pomeroy, R. J. Russell, J. P. Arbuthnott, and D. C. Coleman. 1989. Enterotoxin

- production by *Staphylococcus aureus* isolates from cases of septicaemia and from healthy carriers. *J. Med. Microbiol.* **28**:163-172.
11. Janeway, C. A., J. Chalupny, P. J. Conrad, and S. E. Buxser. 1988. An external stimulus that mimics Mls locus responses. *J. Immunogenetics* **15**:160-166.
 12. Janeway, C. A., J. Yagi, P. J. Conrad, M. E. Katz, B. Jones, S. Vroegop, and S. Buxser. 1989. T cell responses to Mls and to bacterial proteins that mimic its behaviour. *Immunol. Rev.* **107**:61-88.
 13. Kappler, J., B. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrell, D. N. Posnett, Y. Choi, and P. Marrack. 1989. V β -specific stimulation of human T cells by staphylococcal toxins. *Science* **244**:811-813.
 14. Marrack, P., M. Blackman, E. Kushnir, and J. Kappler. 1990. The toxicity of staphylococcal enterotoxin B in mice is mediated by T cells. *J. Exp. Med.* **171**:455-464.
 15. Marrack, P., and J. Kappler. 1990. The staphylococcal enterotoxins and their relatives. *Science* **248**:705-711.
 16. Mathis, D. J., C. Benoist, V. E. Williams II, M. Kanter, and H. O. McDevitt. 1983. Several mechanisms can account for defective E α gene expression in different mouse haplotypes. *Proc. Natl. Acad. Sci. USA* **80**:273-277.
 17. Rizkallah, M. F., A. Tolaymat, J. S. Martinez, P. M. Schlievert, and E. M. Ayoub. 1989. Toxic shock syndrome caused by a strain of *Staphylococcus aureus* that produces enterotoxin C but not toxic shock syndrome toxin-1. *Am. J. Dis. Child.* **143**:848-849.
 18. Sant, A. J., N. S. Braunstein, and R. N. Germain. 1987. Predominant role of amino-terminal sequences in dictating efficiency of class II major histocompatibility complex $\alpha\beta$ dimer expression. *Proc. Natl. Acad. Sci. USA* **84**:8065-8069.
 19. Robinson, J. H., M. C. Atherton, J. A. Goodacre, M. Pinkney, H. Weightman, and M. A. Kehoe. Submitted for publication.
 20. Robinson, J. H., M. C. Atherton, and C. G. Brooks. Submitted for publication.
 21. Vroegop, S. M., and S. E. Buxser. 1989. Cell surface molecules involved in early events in T-cell mitogenic stimulation by staphylococcal enterotoxins. *Infect. Immun.* **57**:1816-1824.
 22. White, J., A. Herman, A. M. Pullen, R. Kubo, J. W. Kappler, and P. Marack. 1989. The V β -specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* **56**:27-35.
 23. Yagi, J., J. Baron, S. Buxser, and C. A. Janeway. 1990. Bacterial proteins that mediate the association of a defined subset of T cell receptor:CD4 complexes with class II MHC. *J. Immunol.* **144**:892-901.