

REGULATORY MECHANISMS IN CELL-MEDIATED IMMUNE RESPONSES

Role of I-J and I-C Determinants in the Activation of H-2I and H-2K/D Alloantigen-specific Suppressor T Cells*

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We have previously described the activation of a population of mixed leukocyte response suppressor T cells (MLR Ts)¹ by H-2-incompatible leukocytes. Reexposure of such cells to priming H-2 alloantigens in vitro triggers the release of a suppressor factor (MLR TsF) that prohibits alloantigen-induced T cell proliferation in the mixed leukocyte response (MLR) (1). Activated MLR Ts and MLR TsF characteristically express I-C alloantigens (2, 3); moreover I-C determinants restrict effective suppressor-responder cell interactions (4). Recent investigations have centered on the specific and perhaps unique requirements for the differentiation and proliferation of H-2 antigen-reactive MLR Ts.

Two previous observations suggest that the MLR Ts population includes two general Ts subsets that recognize either class I (H-2K or H-2D) or class II (H-2I) alloantigens. Partial and additive TsF activity is elicited by individual H-2K and H-2D, as well as H-2I antigen-specific restimulation of an MLR Ts population primed against an entire H-2 haplotype difference (5). In addition, immunoadsorbent analyses indicate that MLR TsF forms complexes with shed stimulator cell antigens in the in vitro restimulation culture, and that these complexes, acting in an alloantigen-nonspecific fashion, represent the major suppressive species in MLR TsF preparations (S. Rich, manuscript in preparation). MLR TsF from Ts primed and restimulated against an entire H-2 complex difference can be isolated into H-2K, D, and I antigen-bound fractions by adsorption to insolubilized antibodies specific for individual stimulator H-2 region antigens. Together these data are consistent with a complex repertoire of H-2 class I and II antigen-specific MLR Ts and TsF.

The present studies examine the role of individual H-2I subregion determinants in the activation of MLR Ts primed to antigens of the entire H-2I region, and their possible expression on those stimulator cells required to trigger primed

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¹ Abbreviations used in this paper: C', complement; CTL, cytotoxic T lymphocyte; FCS, fetal calf serum; IL-2, interleukin 2; LISM, low ionic strength medium; MEM, minimum essential medium; MLR, mixed leukocyte response; MLR Ts, MLR suppressor T cell; MLR TsF, MLR suppressor T cell factor; TdR, thymidine; Th, helper T cell; TNP, trinitrophenyl hapten; Ts, suppressor T cell; TsF, suppressor T cell factor.

H-2K- or D-specific MLR Ts. The following data suggest that I-J and I-C are together the predominant if not exclusive triggering H-2 alloantigens for the H-2I-primed MLR Ts subpopulation. Moreover, these data demonstrate that effective triggering of class I antigen-specific MLR Ts requires not only recognition of the allogeneic priming H-2K or D antigen, but in addition the concomitant recognition of Ts-syngeneic I-C determinants. These findings imply that I-C⁺ as well as I-J⁺ antigen-presenting cells may play unique roles in the activation of H-2 alloantigen-reactive suppressor T cells.

Materials and Methods

Mice. B10.AM, B10.HTG, B10.HTT, B10.OH, B10.A(3R), B10.A(4R), and A.AL mice were purchased from Dr. Chella David, Mayo Clinic, Rochester, MN. Mice bred in our laboratory included A.TH, A.TL, and B10.MBR from breeding stock provided by Dr. Ellen Vitetta, University of Texas Southwestern Medical School, Dallas, TX; B10.S and B10.Q, from breeding stock obtained from Dr. Jack Stimpfling, McLaughlin Research Institute, Great Falls, MT; and (C57BL/6J × B10.BRSgSnJ)F₁, from breeding stock purchased from The Jackson Laboratory, Bar Harbor, ME. All other strains were purchased from The Jackson Laboratory. Male mice 6–12 wk old were routinely used.

Antisera. Monoclonal antibodies used in these experiments were obtained from the following hybridoma preparations: 10.2.16 supernate, anti-I-A^k(Ia.17), Salk Institute Cell Distribution Center, La Jolla, CA; 14.4.4S supernate, anti-I-E^k(Ia.7), American Type Culture Collection (ATCC), Rockville, MD; and WF8.C12-8 ascites, anti-I-J^k, and WF18.2815 ascites, anti-I-J^d, generously provided by Dr. Carl Waltenbaugh, Northwestern University Medical School, Chicago, IL. Conventional antisera included anti-I-C^k, MC-92 (B10.2R × C3H.OL anti-B10.AM) and anti-I-C^d, MC-117 (BSVS × B10.AM anti-B10.A(2R)), kindly provided by Dr. Chella David, Mayo Clinic. All antibody preparations have been characterized for H-2 subregion specificity and effective titer by microcytotoxicity and/or functional assays.

Preparation of MLR Ts and Supernates. Mice were injected with 4×10^7 allogeneic spleen cells in the hind footpads. 4 d later primed spleen cells containing MLR Ts were depleted of dead cells by fractionation with low ionic strength medium (LISM) (6), depleted of erythrocytes with Tris-NH₄Cl buffer, and were cocultured for 24 h with equal numbers of mitomycin C-treated ($50 \mu\text{g/ml}$, 30 min at 37°C; Sigma Chemical Co., St. Louis, MO) stimulator spleen cells at a total cell concentration of $2 \times 10^7/\text{ml}$ in supplemented Eagle's minimal essential medium (MEM) containing 2% fetal calf serum (FCS). Supernates containing MLR TsF were collected by centrifugation of MLR Ts cultures at 2,500 rpm for 10 min and were generally used immediately. Control supernates were obtained from cocultures of unprimed spleen cells of the relevant MLR Ts strain and mitomycin C-treated syngeneic stimulator cells. Primed MLR Ts are designated in the text as the MLR Ts strain followed by a superscript indicating the allogeneic priming strain (e.g., A.TH^{TL} indicates A.TH cells primed with A.TL cells).

Antibody and Complement (C') Depletion of MLR Ts Stimulator Cells. Viable spleen cells prepared by LISM and Tris-NH₄Cl buffer treatment were incubated at $2 \times 10^7/\text{ml}$ with monoclonal antibodies at a usual final dilution of 1:1,000, or with conventional antisera at a final dilution of 1:10, at 4°C for 30 min and washed once. They were resuspended at $2 \times 10^7/\text{ml}$ in 1:10 prescreened young rabbit serum, obtained through the generosity of Dr. Robert Baughn, Veterans Administration Medical Center, Houston, TX, and were incubated for 45 min at 37°C, with mitomycin C incorporated during the last 30 min. Treated cells were washed three times, dead cells were removed by LISM treatment after monitoring specific lysis by trypan blue staining, and remaining viable cells were resuspended as above for culture with MLR Ts. Stimulator cell incubations with anti-I-C sera were extended to 45 min at 4°C, and subsequent complement treatment was extended to 90 min.

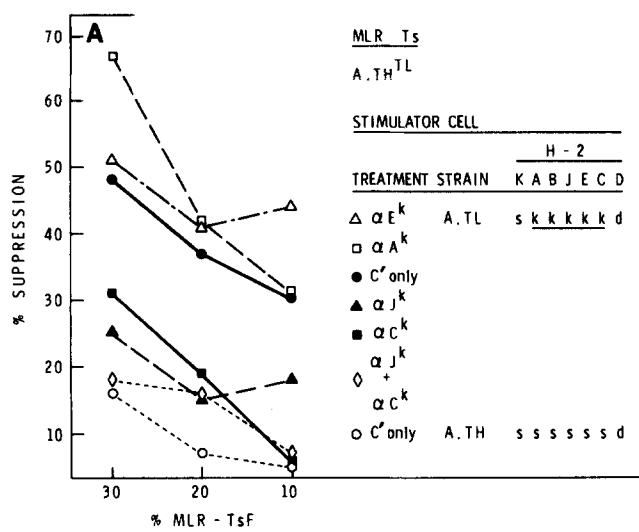
Assay of MLR TsF in MLR Cultures. MLR cultures in a final volume of 0.2 ml were

established in 96-well microtiter plates with 10^6 of each of responder and mitomycin C-treated stimulator cells in supplemented MEM with 10% FCS and 0.1 ml of medium, control factor, or MLR TsF in multiple concentrations. MLR responder cell strains were always H-2-syngeneic with the MLR TsF strain. MLR cultures were incubated at 37°C in an atmosphere of 10% CO₂, 83% N₂, and 7% O₂ for 72 h, with 1 μ Ci [³H]thymidine ([³H]TdR) (sp act 2 Ci/mmol; New England Nuclear, Boston, MA) added during the final 18 h. Cultures were harvested onto glass fiber filters using a MASH II apparatus (M. A. Bioproducts, Walkersville, MD) and processed for liquid scintillation counting. Data are expressed as Δ cpm \pm SEM, representing mean cpm of triplicate cultures with stimulator cells allogeneic to the responder cell strain minus mean cpm of cultures with syngeneic stimulator cells. SEM of Δ cpm were calculated by the formula for the propagation of errors. Percent MLR suppression was calculated as follows: $[1 - (\Delta\text{cpm of MLR with MLR TsF}) / (\Delta\text{cpm of MLR with medium or control factor})] \times 100$. Results were analyzed with a two-tailed Student's *t* test. MLR TsF effects described as suppressive were significantly different from control factor effects at *P* values of <0.05 – <0.001 .

The MLR stimulatory activity of spleen cells depleted of various Ia⁺ subpopulations, and used to activate MLR Ts, was monitored in primary MLR modified to provide optimal specific responses to single H-2K and D as well as I region differences (Figs. 1 and 2 B). 4×10^5 viable splenic responder cells and 5×10^5 viable mitomycin C-treated splenic stimulator cells were cultured in 0.2 ml of Click's medium (7) modified exactly according to Corradin et al. (8) in 5% CO₂ for 120 h, including a final 18 h [³H]TdR pulse of 1 μ Ci/culture. Stimulator cells were aliquots of those control or antibody and C'-treated spleen cells prepared as described above to stimulate MLR TsF from H-2-primed MLR Ts.

Results

Expression of H-2I Subregion Alloantigens on Stimulator Cells Required to Trigger H-2I Alloantigen-primed MLR Ts. Initial studies examined individual H-2I subregion alloantigens that have the particular capacity to trigger TsF production by MLR Ts primed to entire H-2I region antigenic differences. A.TH spleen cells were primed with I region disparate A.TL splenocytes, and restimulated with A.TL stimulators that were untreated or depleted of subpopulations bearing various H-2I subregion antigens by subregion-specific antibody and C' lysis (Fig. 1A). Activated H-2K, D, or I region-specific Ts subsets typically generate MLR TsF that suppresses MLR 30–45% in contrast to 85% or greater suppression after activation of all MLR Ts subsets (5). In the A.TH anti-A.TL strain combination, MLR Ts priming and restimulation potentially occurs to alloantigens encoded by all I subregions and a characteristic profile of suppression is seen. Depletion of stimulator cells expressing either A^k or E^k antigens failed to reduce activation of H-2I^k-primed MLR Ts, but instead routinely enhanced MLR TsF activity to a modest extent. In contrast, stimulators depleted of either J⁺ or C⁺ cells failed to support MLR TsF production. To determine if the stimulatory I-J and I-C determinants were expressed on a single or on distinct cell populations, aliquots of A.TL stimulator cells treated separately with anti-J^k plus C' and anti-C^k plus C' were combined in equal proportion and used to activate the H-2I^k-primed MLR Ts. However, TsF production was not restored by this stimulator cell mixture, which suggests that a single I-J⁺, I-C⁺ spleen cell population possessed the predominant activating properties for H-2I-primed MLR Ts. Although priming and restimulation of A.TH^{TL} may theoretically include priming to class I Qa-1^b alloantigens (9), I-A^J stimulator cells were also required to restimulate H-2I^s-specific A.TL^{A.SW} anti-A.TH MLR Ts, in which



B STIMULATION OF PRIMARY MLR BY MLR Ts STIMULATORS

| MLR RESPONDER | STRAIN | STIMULATOR CELL | | | | | | TREATMENT | MLR RESPONSE | |
|---------------|--------|-----------------|---|---|---|---|---|--------------|--------------|-----------|
| | | H - 2 | | | | | | | Δ CPM | % CONTROL |
| A.TH | A.TL | s | k | k | k | k | d | C' only | 104,154 | 100 |
| | | | | | | | | αA^k | -1585 | -2 |
| | | | | | | | | αJ^k | 89,673 | 86 |
| | | | | | | | | αE^k | 11,539 | 11 |
| | | | | | | | | αC^k | 118,894 | 114 |

FIGURE 1. Expression of H-2I subregion alloantigens on stimulator cells required to activate H-2I alloantigen-primed MLR Ts. (A) A.TL-primed A.TH MLR Ts were restimulated for MLR TsF production with A.TL stimulator cells remaining after treatments indicated on figure. Control stimulators were syngeneic A.TH cells treated with C' only (O). Underlined subregions of the stimulator H-2 designation indicate those subregions shared by the priming and restimulating strains and disparate to the MLR Ts strain. Data are from a representative experiment, using a B10.S anti-B10 assay MLR. (B) Aliquots of the antibody plus C'-treated A.TL stimulator cells used to trigger MLR TsF production in A were also used as stimulators of MLR with unprimed A.TH responder cells, under conditions described in Materials and Methods.

Qa-1 priming does not occur (data not shown). Thus under the conditions of priming and restimulation used in these studies, activation by A⁻/E⁻, J⁺/C⁺ stimulator cells identified in the A.TH anti-A.TL MLR Ts combination reflects the requirements for triggering H-2I antigen-specific MLR Ts, but not H-2 class I antigen-specific Ts, whose requirements will be shown below to be distinct.

Aliquots of each of the antibody and C'-treated spleen cell preparations used above to trigger H-2I-primed MLR Ts were also used to stimulate primary MLR responses of A.TH spleen cells to obtain functional evidence of the depletion of A⁺ and E⁺ subsets (Fig. 1 B). A⁻ or E⁻ cells retained little or no MLR stimulatory activity, although from the above studies the remaining cells clearly stimulated MLR Ts. In contrast, the MLR-stimulating properties of J⁻ or C⁻ spleen cells

were largely unaffected, although these same populations were ineffective stimulators of H-2I-primed MLR Ts. These data thus support the interpretation that H-2I-primed MLR Ts do not recognize conventional I-A- or I-E-encoded class II alloantigens directly, and do not require A⁺ or E⁺ alloantigen-presenting cells. Rather, stimulator cells bearing I-J as well as I-C determinants are required to trigger MLR Ts primed to I region alloantigens.

H-2I Subregion Alloantigens That Activate H-2I Antigen-primed MLR Ts. To clarify whether allogeneic I-J and I-C determinants on a common alloantigen-presenting cell are independently recognized by distinct MLR Ts subpopulations or if in fact only one of these molecules is the triggering stimulus, MLR TsF production was assessed using primed MLR Ts/stimulator cell combinations that provided only isolated I-J or I-C subregion differences (Table I). B10.BR-primed B10.A(3R) MLR Ts, restimulated only by the priming I-J^k on B10.A(5R), produced significant MLR TsF (experiment 1). Removal of I-J⁺ stimulator cells prevented TsF generation, while removal of I-E⁺ cells had no effect, consistent with the previous observation that the I-J antigen recognized by MLR Ts is not coexpressed on A⁺/E⁺ cells. (C57BL/6 × B10.BR) F₁ MLR Ts primed and restimulated only by allogeneic I-C^d determinants similarly generated MLR TsF (experiment 2). TsF production was sensitive to depletion of stimulator cells

TABLE I
H-2I Alloantigen Requirements for Activation of H-2I Antigen-primed MLR Ts

| Experiment | MLR TsF culture | | | | | | | | | | MLR assay [†] | | |
|------------|---------------------------------------|------------------------|------------------|----------|----------|----------|----------|----------|---|---------------------|------------------------|-------------|---------------------|
| | MLR Ts* | Strain | Stimulator cell | | | | | | | | Treatment [‡] | Δ cpm ± SEM | Percent suppression |
| | | | H-2 [†] | | | | | | | | | | |
| K | A | B | J | E | C | D | | | | | | | |
| 1 | B10.A(3R) | B10.A(3R) | b | b | b | b | k | d | d | — | 13,991 ± 1,004 | — | |
| | B10.A(3R) ^{BR} | B10.A(5R) | b | b | b | <u>k</u> | k | d | d | — | 9,094 ± 571 | 35 | |
| | | | | | | <u>k</u> | k | d | d | anti-E ^k | 9,718 ± 861 | 31 | |
| | | | | | | <u>k</u> | k | d | d | anti-J ^k | 13,138 ± 588 | 6 | |
| 2 | (B6 × BR)F ₁ | B6 × BR F ₁ | k/b | k/b | k/b | k/b | k/b | k/b | k/b | — | 11,426 ± 1,247 | — | |
| | (B6 × BR)F ₁ ^{2R} | B10.A(2R) | k | k | k | k | k | <u>d</u> | b | — | 7,655 ± 501 | 33 | |
| | | | | | | | | <u>d</u> | b | anti-A ^k | 7,884 ± 312 | 31 | |
| | | | | | | | | <u>d</u> | b | anti-J ^k | 10,169 ± 733 | 11 | |
| | | | | | | | | <u>d</u> | b | anti-C ^d | 10,398 ± 1,061 | 9 | |
| | | | | | | | <u>d</u> | b | anti-J ^k + anti-C ^d | 10,741 ± 1,247 | 6 | | |
| 3 | B10.A(5R) | B10.A(5R) | b | b | b | k | k | d | d | — | 13,060 ± 650 | — | |
| | B10.A(5R) ^{MBR} | B10.A(5R) | b | b | b | k | k | d | d | — | 12,146 ± 1,115 | 7 | |
| | | B10.MBR | b | <u>k</u> | <u>k</u> | k | k | k | k | — | 6,008 ± 287 | 54 | |
| | | B10.A | k | <u>k</u> | <u>k</u> | k | k | <u>d</u> | <u>d</u> | — | 11,885 ± 1,384 | 9 | |
| | | B10.A(4R) | k | <u>k</u> | <u>b</u> | b | b | b | b | — | 12,276 ± 1,135 | 6 | |
| | | B10.HTT | s | s | s | s | k | k | d | — | 9,272 ± 408 | 29 | |
| | | B10.OH | d | d | d | d | d | <u>d</u> | <u>k</u> | — | 7,967 ± 759 | 39 | |

* MLR Ts were prepared from spleens of animals that had been injected into the footpads 96 h previously with 40×10^6 spleen cells of the strains indicated by superscript designation.

[†] Underlined subregions of the stimulator strain H-2 designation indicate those subregions shared between the priming and restimulation strains and distinct from the MLR Ts strain.

[‡] Stimulator cells were untreated or treated with antibody and C' before addition to MLR TsF cultures. In experiment 2, the final experimental group (anti-J^k + anti-C^d) used stimulator cells comprised of equal numbers of cells independently treated with anti-J^k and anti-C^d antibodies.

[§] MLR cultures to assay MLR TsF were constructed as follows: experiment 1, B10.A(3R) anti-B10.S; experiment 2, (B6 × BR)F₁ anti-B10.S; experiment 3, B10.A(5R) anti-B10.S. Data represent responses of MLR receiving 20% final volume MLR TsF. The data are from representative experiments.

bearing the priming allogeneic I-C^d determinant as well as MLR Ts-syngeneic I-J^k; a mixture of C^d- and J^k-depleted stimulator populations did not restore MLR Ts activation, suggesting again that the I-J and I-C determinants recognized by MLR Ts exist primarily on a common cell population. Thus, independent recognition of either I-J or I-C allogeneic determinant in the absence of other allogeneic H-2 molecules is sufficient to trigger the appropriate MLR Ts subpopulation to TsF production. In contrast, restimulation only by priming I-A^k/B^k or I-A^k alloantigens alone failed to generate MLR TsF from B10.MBR-primed B10.A(5R) MLR Ts, although priming B10.MBR cells that also express allogeneic I-C^k and H-2D^k were effective, as were B10.HTT and B10.OH, which displayed only I-C^k or H-2D^k, respectively (experiment 3). These data suggest that H-2I-specific MLR Ts are comprised of two distinct alloreactive populations that independently recognize I-J or I-C alloantigens, while T cells that bind I-A and express MLR Ts activity are not evident.

Expression of H-2I Determinants on Stimulator Cells Required to Trigger H-2 Class I-specific MLR Ts. MLR Ts primed to allogeneic H-2K or D antigens are triggered by stimulator cells that express the priming class I molecules but that are otherwise H-2-syngeneic with the MLR-Ts (5). Since class I molecules are ubiquitously expressed, it was of interest to determine if the appropriate H-2K/D determinant is the sole triggering element and thus effective regardless of the particular cell subset upon which it is expressed, or if additional differentiation determinants, including H-2I subregion molecules, characterize cells with distinctive MLR Ts-stimulating qualities. To test the latter possibility, stimulator cells bearing the priming H-2K or D antigen were treated with H-2I subregion-specific antibodies and C', and the remaining viable cells were used to trigger TsF production by H-2K or D antigen-primed MLR Ts. In Fig. 2A, B10-primed B10.BR MLR Ts were restimulated with B10.AM spleen cells, in all cases bearing only the priming allogeneic H-2D^b determinant but variously depleted of I-A⁺, I-E⁺, I-J⁺, or I-C⁺ subpopulations. Removal of I-A- or I-E-bearing cells did not decrease MLR Ts activation but instead somewhat enhanced TsF production. Removal of I-J⁺ stimulators minimally reduced TsF activity. In contrast, H-2D^b-bearing cells depleted only of the I-C⁺ subpopulation were deficient in their capacity to elicit TsF production by H-2D^b-primed MLR Ts.

To establish that class I alloantigen expression by remaining I-C⁻ cells was not impaired, aliquots of the untreated or antibody and C'-treated B10.AM stimulator cells were also used as stimulators of primary H-2D^b-specific MLR with B10.BR responder cells (Fig. 2B). In accord with the findings of others (10-12), I-A⁻ as well as I-E⁻ stimulators lacked the capacity to promote D^b-specific proliferation, although the above studies indicate they clearly expressed H-2D^b and were effective MLR Ts stimulators. In contrast, H-2D^b-specific proliferation was not diminished but rather was somewhat enhanced by removal of either I-J⁺ or I-C⁺ cells. Therefore failure of the I-C⁻ population to promote effective D^b-specific MLR Ts activation did not result from inadequate H-2D^b expression.

Restimulation requirements for H-2K-primed MLR Ts follow a similar pattern (Fig. 3). Thus both I-A⁻ and I-J⁻ H-2K^s-bearing A.TL spleen cells fully restimulated A.SW-primed A.AL MLR Ts, while I-C⁻ cells lacked the capacity to optimally trigger K^s-specific Ts.

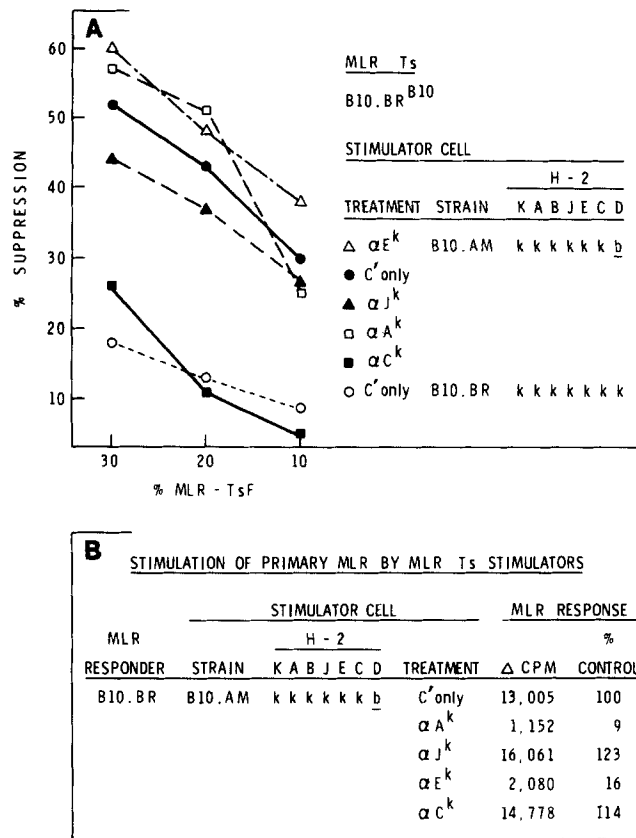


FIGURE 2. Expression of H-2I determinants on stimulator cells required to trigger H-2D-specific MLR Ts. (A) B10-primed B10.BR MLR Ts were restimulated for MLR TsF production with B10.AM stimulator cells remaining after treatments indicated on figure. Control stimulators were syngeneic B10.BR cells treated with C' only (O). Underlined subregions of the stimulator H-2 designation indicate those subregions shared by the priming and restimulating strains and distinct from the MLR Ts strain. Data are from a representative experiment using a B10.BR anti-B10.S assay MLR. (B) Aliquots of the antibody plus C'-treated B10.AM stimulator cells used to trigger MLR TsF production in A were also used as stimulator cells in a primary anti-D^b MLR with unprimed B10.BR responder cells under conditions described in Materials and Methods.

I-C-restricted Activation of Class I-specific MLR Ts. In the preceding experiments triggering of allogeneic H-2K- or D-specific MLR Ts was prevented by removal of stimulator cells bearing I-C determinants that were syngeneic with the MLR Ts strain. However it was not clear if an I-C molecule of the MLR Ts haplotype was specifically required or if restimulation instead required the activity of I-C⁺ cells, regardless of the particular I-C allelic product expressed. Therefore H-2^b alloantigen-primed MLR Ts were cultured with stimulator cells bearing the priming H-2D^b alloantigen and I-C determinants syngeneic or disparate with the MLR Ts I-C haplotype (Table II). Only cells bearing both H-2D^b and the MLR Ts-syngeneic I-C^k (experiment 1) or I-C^d (experiment 2) determinants successfully triggered TsF production, while those stimulators expressing either an inappropriate H-2D allele or Ts-disparate I-C determinant were ineffective.

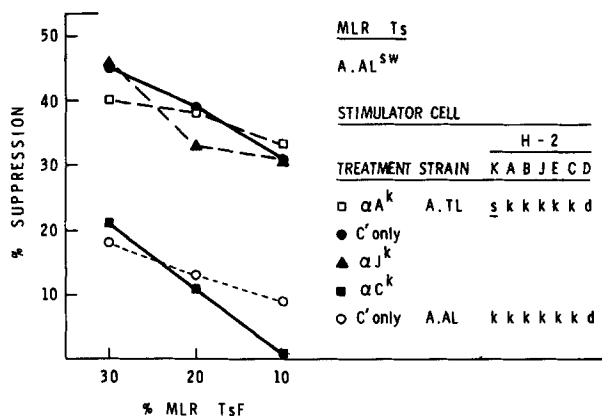


FIGURE 3. Expression of H-2I determinants on stimulator cells required to trigger H-2K-specific MLR Ts. A.AL MLR Ts were primed with A.SW(H-2^b) spleen cells and restimulated for MLR TsF production with A.TL stimulator cells remaining after treatments indicated on figure. Control stimulators were syngeneic A.AL cells treated with C' alone (O). Underlined subregions of the stimulator H-2 designation indicate subregions shared by the priming and restimulating strains and distinct from the MLR Ts strain. Data are from a representative experiment using a B10.BR anti-B10 assay MLR. Aliquots of treated MLR Ts stimulator populations were also used to stimulate primary MLR with unprimed A.AL spleen cells, and the following Δ cpm values were obtained with A.TL stimulators treated as indicated: C' control, 15,559; anti-A^k, 1,910; anti-J^k, 26,886; and anti-C^k, 16,036.

TABLE II
I-C-restricted Activation of Class I-specific MLR Ts

| Experiment | MLR TsF culture | | | | | | | | MLR assay [§] | | | | |
|------------|-----------------------|------------------------------|----------|----------|----------|----------|----------|----------|------------------------|---------------------|--------------------|-----------------|---|
| | MLR Ts* | Stimulator Cell [‡] | H-2 | | | | | | Δ cpm \pm SEM | Percent suppression | | | |
| | | | Strain | K | A | B | J | E | | | C | D | |
| 1 | B10.BR | B10.BR | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | 14,828 \pm 1,046 | — | | |
| | B10.BR ^{B10} | B10.AM | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <u>b</u> | 10,231 \pm 922 | 31 | |
| | | B10.A(2R) | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <u>b</u> | 13,790 \pm 558 | 7 | |
| | | B10.AKM | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <u>q</u> | 13,641 \pm 1,393 | 8 | |
| | | B10.BR | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | 13,938 \pm 786 | 6 | |
| 2 | B10.A | B10.A | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | 7,038 \pm 744 | — | |
| | B10.A ^{B10} | B10.A(2R) | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <u>b</u> | 4,996 \pm 412 | 29 | |
| | | B10.AM | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <u>b</u> | 6,404 \pm 712 | 9 | |
| | | B10.HTG | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <u>b</u> | 4,645 \pm 402 | 34 | |
| | | B10.D2 | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <i>d</i> | <u>d</u> | 6,686 \pm 672 | 5 | |
| | | B10.A | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>k</i> | <i>d</i> | <i>d</i> | <i>d</i> | 6,756 \pm 638 | 4 |

* MLR Ts preparation as Table I.

‡ Underlined subregions of the stimulator strain H-2 designation indicate those subregions shared by the priming and restimulating strains and distinct from the MLR Ts strain. Italicized subregions are those that are shared between the MLR Ts and restimulating strains.

§ MLR TsF was assayed in MLR as in Table I. MLR cultures to assay MLR TsF used the following responder/stimulator cell combinations: experiment 1, B10.BR anti-B10.S; experiment 2, B10.A anti-B10.S.

Thus B10-primed B10.A MLR Ts responded to B10.A(2R) cells, which expressed H-2D^b and were otherwise syngeneic with B10.A MLR Ts, and to B10.HTG cells, which similarly displayed H-2D^b and Ts-syngeneic I-C^d but which were otherwise distinct from the Ts haplotype (experiment 2). In the latter case Ts activation clearly resulted from H-2D^b restimulation rather than from primary recognition of allogeneic K-end H-2^d molecules, since B10.D2 (H-2^d) stimulators were not effective. B10.AM stimulators were similarly ineffective although they differ from MLR Ts only in the I-C subregion, while presenting the correct priming H-2D^b molecule. Finally, H-2D^b specificity was illustrated by failure of B10.AKM(H-2D^q) to trigger H-2D^b-primed B10.BR MLR Ts (experiment 1). Thus both serologic and genetic studies suggest that MLR TsF production by H-2 class I antigen-primed Ts is dependent upon recognition of self-I-C determinants that occurs concomitantly with recognition of the priming class I alloantigen, and that other H-2I-encoded determinants do not fulfill the self-recognition function.

Discussion

These studies have identified the H-2-encoded molecules that characterize those stimulator cells required for the activation of suppressor T cells primed to allogeneic H-2K and D, or H-2I alloantigens. The serologic and genetic analyses reported here each suggest that allogeneic I-J- and I-C-encoded molecules are together the major and perhaps the sole alloantigenic triggers of MLR Ts primed to disparate H-2I region determinants. Moreover, the relevant I-J and I-C determinants appear to be coexpressed on a single antigen-presenting cell subpopulation that is used in common by I-J- and I-C-specific MLR Ts. In contrast, conventional allogeneic I-A and I-E molecules appear to be neither essential nor sufficient triggering elements for H-2I-primed MLR Ts. Finally, as a population, those stimulator cells required to trigger MLR Ts primed to class I H-2K or D alloantigens must express not only the priming class I determinant, but in addition MLR Ts-syngeneic I-C alloantigens. Thus, recognition of self-I-C molecules may constitute an important feature of the triggering, and by implication, priming process of class I-specific MLR Ts.

I-J molecules have been amply documented for their characteristic display on cells and soluble products associated with antigen-specific T cell-mediated suppression (13). In addition, accumulating evidence now strongly suggests that Ts-syngeneic I-J determinants function as restriction elements for recognition of nominal antigen by Ts, particularly those representing late-stage participants in multicellular suppressor pathways (14, 15). T cell recognition of allogeneic I-J determinants appears to stimulate nonspecific suppressive allogeneic effects. Thus Bromberg et al. (16) demonstrated that T cell recognition of allogeneic I-J on Ts that have also bound suboptimal doses of TNP activated full suppressor activity of TNP-specific Ts. T cells primed and restimulated by allogeneic I-J determinants also nonspecifically depress proliferative (17) and delayed hypersensitivity (18) responses of T cells interacting with other allogeneic determinants on a common stimulator cell surface. As documented in the present report, MLR TsF is produced by Ts responsive to allogeneic I-J molecules, and thus is a potential mediator of such I-J-induced allogeneic effects.

I-C alloantigen involvement in T_s responses is less widely described than that of I-J, and the role of allogeneic as well as syngeneic I-C determinants in activation of MLR T_s populations is of particular interest. These data clearly demonstrate that there exists a population of MLR T_s that are solely responsive to allogeneic I-C-encoded determinants, and that I-C- and I-J-specific MLR T_s constitute the two major T_s populations responsive to allogeneic I region antigens. I-C- rather than I-J-triggered allogeneic interactions induce suppression of T-independent B cell responses to dinitrophenylated polyacrylamide beads (19), and may possibly occur via the soluble mediators that possess MLR T_sF activity.

The role of MLR T_s-syngeneic I-C determinants in the activation of H-2K- and D-specific MLR T_s offers some insight into their triggering requirements. I-C recognition is supported by two observations. First, cytolytic removal of I-C⁺ cells from the stimulator population prohibits optimal MLR T_s activation, although cells bearing the appropriate H-2K or D determinant are clearly present as reflected in the MLR control studies. Second, restimulation of allogeneic H-2K- or D-primed MLR T_s fails if the stimulator population lacks either the priming class I alloantigen or the MLR T_s-syngeneic I-C molecule, as determined by the H-2 genetic composition of the stimulator cell. In each instance stimulators that coexpress I-J determinants appear not to be used. Both observations are consistent with the interpretation that the expression of primed H-2K- or D-specific MLR T_s activity requires the additional recognition of host I-C determinants encountered during the priming process.

The apparent necessity for self-I-C recognition may have two functional interpretations. I-C molecules may trigger a cell population distinct from MLR T_s to provide a requisite soluble proliferative or differentiative signal to primed MLR T_s subsequent to H-2K or D antigen binding. In this sense the I-C-reactive cell would facilitate class I-specific MLR T_s activation much the same as I-A- or I-E-specific Th promote class I-specific cytotoxic T lymphocyte (CTL) activation via interleukin (IL) production (20, 21). This model would predict that a population of cells strictly specific for syngeneic I-C is activated and expanded during priming by fully H-2-allogeneic cells, and that soluble mediators generated during self-I-C recognition would promote the activation of class I antigen-specific MLR T_s. In general support of this possibility, autoreactive class II MHC antigen-specific T cells are generated in the course of Th activation by allogeneic Ia (22) or conventional antigen (23, 24), and release of IL-2 follows syngeneic I-A recognition by IL-2-producing T cells (25). Alternatively, self-I-C molecules may participate directly in H-2K- or D-specific MLR T_s activation by serving as restricting elements in the recognition of associated allogeneic class I molecules. Such an interpretation also requires that initial priming involve presentation of shed allogeneic class I molecules on I-C⁺ T_s host cells, since the original priming cells are fully H-2 allogeneic. Shedding of MHC determinants (26) and reorientation and recognition of these shed alloantigens on host cells have been documented by others (27–29).

Analyses of triggering requirements for class I antigen-specific Th cells provide largely analogous findings (10–12, 21, 28). In contrast, however, expression of syngeneic Ia (I-A or I-E) determinants on the stimulator population, rather than I-J/I-C, is requisite to Th triggering by allogeneic class I antigens.

The significance of self-I-C molecules to class I antigen-specific Ts is further supported by the general observation that I-C involvement in Ts responses has been defined almost exclusively in those responses that involve reactivity to allogeneic (3, 4, 30–32) or modified self (33, 34) class I alloantigens. In an exceptional example, the generation of I-J⁺ I-C⁺ Ts that produce I-C-bearing and I-C-restricted TsF has been reported in the course of granulomatous host response in chronic schistosomiasis (35, 36). Brondz et al. (30) demonstrated that class I-specific Ts bind to fully allogeneic cell monolayers, i.e., in the absence of self-I-C. However, restimulation of these eluted class I-specific Ts occurred in the presence of Ts-syngeneic I-C determinants, thus fulfilling the potential requirement for self-I-C recognition in triggering per se. Diminution of MLR proliferation (17) and of delayed hypersensitivity (18) to H-2D antigens reportedly accompanies recognition of allogeneic I-J determinants on a common stimulator cell surface. Although the present studies have not analyzed responses to allogeneic, rather than syngeneic I-J or I-C molecules in activation of H-2K/D-specific MLR Ts, clearly allogeneic I-J or I-C are not required to achieve class I-specific MLR Ts activation.

The absence of T cells that bind I-A or I-E antigens and subsequently mediate typical MLR TsF activity is also suggested by these data. However, it is entirely possible that I-A/E-specific T cells contribute to early phases of MLR Ts activation, perhaps in an inductive capacity, and these would be triggered under the conditions of full H-2 antigen priming used in these studies. In this regard others have reported I-A-specific T cells that participate in suppressive interactions under certain conditions of allogeneic (37, 38) or syngeneic (39) I-A presentation.

Finally, these studies imply that certain Ts use a subset of antigen-presenting cells that are phenotypically and perhaps functionally distinct from those generally used by Th cells, and suggest the existence of activation requirements that are novel in comparison with those required for other H-2 alloantigen-reactive T cells (40–42). Although the precise roles of I-C and I-J molecules and the stimulatory cells that bear them are unknown, their further analysis promises insight into mechanisms of alloantigen recognition and associated triggering signals used by H-2 alloantigen-specific Ts cells.

Summary

The role of individual H-2I subregion determinants in the activation of H-2I alloantigen-primed mixed leukocyte response suppressor T cells (MLR Ts), as well as their possible expression on stimulator cells required to trigger primed H-2K- or D-specific MLR Ts, was addressed in these studies. Both genetic and serologic studies demonstrated that MLR Ts potentially primed to alloantigens encoded by the entire H-2I region were triggered to MLR Ts factor production only by stimulator cells bearing the priming I-J and/or I-C, but not I-A or I-E alloantigens. The relevant I-J and I-C determinants were demonstrated on a single antigen-presenting cell population that is used in common by independent I-J-specific and I-C-specific MLR Ts. Unexpectedly, the stimulator cell population necessary to trigger MLR Ts primed to class I H-2K or D alloantigens expressed not only the priming class I determinant, but in addition, I-C alloantigens

syngeneic with the MLR Ts haplotype. Stimulator populations bearing the appropriate H-2K or D alloantigen but serologically depleted of I-C⁺ cells or genetically constructed to display MLR Ts-disparate I-C determinants were ineffective stimulators of class I antigen-primed MLR Ts. Thus these data suggest that as allogeneic determinants, I-J- and I-C-encoded molecules are together the major triggering elements for MLR Ts primed to disparate H-2I region determinants. In addition, self-I-C molecule recognition appears to constitute an important feature of the triggering, and by implication, priming process of H-2 class I antigen-specific Ts cells.

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