REGULATORY MECHANISMS IN
CELL-MEDIATED IMMUNE RESPONSES

VIII. Differential Expression of I-Region Determinants by Suppressor Cells and their Targets in Suppression of Mixed Leukocyte Reactions*

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Gene products of the I region of the murine major histocompatibility complex (MHC) play critical roles in focusing precise interactions of regulatory T lymphocytes with other cells of the immune system. Participation of I-region molecules in antigen recognition by subpopulations of helper T cells reflects the possession of receptors specific for I-region products and their function in mediating specific interactions with antigen-presenting cells (1, 2). In addition, primed suppressor T cells, and to some extent helper cells, display distinct I-region-encoded determinants which are consistent with their regulatory commitment (3–9). Because these I-region molecules are also requisite components of certain soluble regulatory T-cell products (10–13), they may have a direct function in mediating appropriate regulatory interactions. The most well developed model of I-region involvement in regulatory T-cell function derives from investigations of antigen-specific T-cell-mediated suppression. Gene products of the I-J subregion are selectively expressed on a class of T cells functionally committed to suppression in a number of antigenically diverse immune responses (4–9, 14). Moreover, molecules encoded by the I-J subregion are components of several T-cell-derived suppressor factors (10–12). These studies have suggested that I-J gene products serve as structures of common regulatory function in responses to a variety of conventional antigens.

In contrast to the apparently general role of I-J molecules in antigen-specific immune suppression, antigen-nonspecific suppression of proliferative responses stimulated by MHC antigens is controlled by genes of a distinct I subregion. Murine mixed leukocyte responses (MLR) are suppressed by a soluble factor (MLR-TsF) from alloantigen-activated T cells (15); suppression requires the presence of molecules

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Abbreviations used in this paper: Con A, concanavalin A; FCS, fetal calf serum; GT-TsF, L-glutamic acid-L-tyrosine suppressor factor; HBSS, Hanks’ balanced salt solution; MHC, major histocompatibility gene complex; MLR, mixed leukocyte reaction; MLR-Ts, mixed leukocyte reaction suppressor T cell; MLR-TsF, mixed leukocyte reaction suppressor T-cell factor; NMS, normal mouse serum; Ts, T suppressor.
encoded by the I-C, rather than I-J subregion (16). MLR-TsF suppression is further restricted to responses of cells derived from strains syngeneic with suppressor cell strains at the I-C subregion (17). Products encoded by the I-C subregion thus appear to provide an additional molecular focus of regulatory T-cell interaction. To further characterize the function of I-C molecules in MLR suppression, we have explored the expression of I-C, as well as other I-region products on the suppressor T cell which is stimulated to produce MLR-TsF. In addition, we have investigated the possible expression and role of I-region determinants on cellular targets of MLR-TsF. Data in this report establish that MLR suppressor T cells possess determinants encoded by the I-C subregion. These molecules, like regulatory I-J determinants, are preferentially expressed on T cells but not B cells. In addition products of the I-J subregion are concomitantly expressed on the same MLR suppressor cell, further generalizing the role of these molecules as functional markers. In contrast, targets of MLR-TsF activity fail to display I-C, or other I-region determinants either as functional components of MLR-TsF receptors or as markers characterizing the target cell.

Materials and Methods

Mice. BALB/c mice were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, Texas. Strains CBA/J, C57BL/6J(B6), and A/J were purchased from The Jackson Laboratory, Bar Harbor, Maine. 6- to 12-wk-old male mice were used.

Antisera. The antisera B10.A(3R) anti-B10.A(5R), (B10.D2 × A.BY)F1 anti-B10.A(5R), B10.HTT anti-B10.S(9R), (B10.S(9R) × A.TFR5)F1 anti-A.TL, B10.S(9R) anti-B10.HTT, and (B10.A(4R) × B10.AM)F1 anti-B10.A(2R) were produced in the Department of Immunology, Mayo Clinic and Medical School, Rochester, Minn. Antisera A.TH anti-A.TL and (A.BY × B10.HTT)F1 anti-A.TL were obtained through the courtesy of the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. All sera, including normal mouse sera (NMS), were preadsorbed with spleen cells of the recipient strain used in serum production, or an H-2-identical strain.

Preparation of T and B Cells for Antiserum Adsorption. Thymocytes and spleen cells were used as T, and T- and B-cell sources, respectively. Nonactivated T cells were obtained by nylon wool fractionation of normal spleen cell suspensions (18). The percentage of T cells in the effluent was &gt;90% as judged by killing with anti-Thy-1.2 antiserum (lot BA002, Litton Bionetics, Kensington, Md.) and complement. Concanavalin A(Con A)-activated thymocytes were prepared by incubating single cell thymocyte suspensions at 10^7 cells/ml in supplemented Eagle’s minimal essential medium (MEM) (19) containing 5% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.) and 2 μg/ml Con A (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). 5-ml cultures in 60-mm dishes were maintained on rocking platforms for 48 h at 37°C in an atmosphere of 10% CO_2, 7% O_2, and 83% N_2. Cultured thymocytes were washed extensively with 0.15 M methyl-α-D-mannoside in Hanks’ balanced salt solution (HBSS) before use in antiserum adsorption. Con A-stimulated thymocytes were generally at least 90% Thy-1.2 positive, 80% viable, and 80% blast forms. Spleen B cells were prepared by incubation of normal spleen cells, selected for viability by treatment with low ionic strength medium (20), with anti-Thy-1.2 serum diluted 1:20 in HBSS and incubated for 45 min at 37°C with rabbit complement (Pel-Freeze Biologicals Inc., Rogers, Ariz.) diluted 1:4 and containing 10 μg/ml DNase (Worthington Biochemical Corp., Freehold, N. J.). Dead cells were removed by low ionic strength medium treatment, and viable cells used for adsorption.

Adsorption of Anti-I Region Sera. 100 μl of antiserum (B10.A(4R) × B10.AM)F1 anti-B10.A(2R) were diluted 1:10 with HBSS with 2% FCS and adsorbed with 2 × 10^6 cells of the adsorbing cell preparations described above for 1 h at room temperature. Adsorbing cells were removed by centrifugation and the supernates were collected for use in cytotoxic assays.

Mixed Leukocyte Reaction Assay. MLR were prepared as previously described (19). Briefly, responder and stimulator cell populations were cultured in equal numbers, 5 × 10^5 viable cells of each in 0.2-ml supplemented Eagle’s MEM with 10% FCS. Stimulator cells (designated
throughout by the subscript m) were treated before addition to MLR with mitomycin C (Sigma Chemical Co., St. Louis, Mo.). DNA synthesis in MLR was assayed by adding 1.0 μCi of tritiated thymidine (sp act 2.0 Ci/mmol; New England Nuclear Corp., Boston, Mass.) to cultures for the final 18 h of a 72- or 96-h incubation period.

Data from individual experiments are expressed as mean counts per minute of three to four replicate cultures with the standard error of the mean. Net counts per minute (E-C) were calculated by subtracting counts per minute of cultures with syngeneic stimulating cells (C) from counts per minute of cultures with allogeneic stimulating cells (E). Percent control MLR response was calculated according to the following formula:

\[
\frac{(E-C) \text{ of MLR with supernate of alloantigen-stimulated cells}}{(E-C) \text{ of MLR with supernate of control cells}} \times 100 = \text{percent control MLR response.}
\]

Preparation of Suppressor and Control Supernates. Normal mice were injected with 4–5 × 10⁷ allogeneic spleen cells into hind footpads. 4 d later allogeneic-activated spleen cells were treated with low ionic strength medium, exposed to antiserum and complement if appropriate, and co-cultured in supplemented Eagle’s MEM with 2% FCS with equal numbers of mitomycin C-treated allogeneic spleen cells of the strain used for in vivo sensitization. Supernates were harvested 24 h later. Control supernates were similarly prepared from co-cultures of normal antiserum-treated or untreated spleen cells with equal numbers of mitomycin C-treated syngeneic cells. Alloantigen-activated suppressor cells or factor are designated by the name of the suppressor cell strain with the sensitizing strain following in superscript, i.e., “BALB/B6” indicates BALB/c cells sensitized and restimulated with B6 cells.

Cytotoxic Treatment of Cells with Alloantisera. Normal or alloantigen-activated spleen cells were pretreated with low ionic strength medium, and incubated at 3 × 10⁷ cells/ml with 1:10 dilution of antiserum or NMS in HBSS with 2% FCS. After incubation for 40 min at 4°C, cells were washed in HBSS and further incubated for 90 min at 37°C with a 1:4 dilution rabbit complement (C). The cells were washed twice and viability determined by trypan blue exclusion. After removal of dead cells, remaining viable cells were cultured either for production of MLR-TsF or as MLR responder cells.

In cytotoxic treatment of cells from 24 h MLR, responder and stimulator cells which had been cocultured in the absence of factors were harvested from microtiter plates and washed twice in HBSS. Aliquots of mixed and control cultures were treated as described above with anti-sera or NMS which had been preadsorbed with cells of the allogeneic stimulator cell strain. After removal of dead cells, remaining viable cells of each treatment group were divided into three portions and exposed to culture medium with 2% FCS or undiluted control or suppressor factors for 40 min at 4°C. Pulsed cells were washed three times with HBSS and replaced at 7 × 10⁵/well in culture wells containing retained washed adherent cells. Cultures were harvested 72 or 96 h after culture initiation.

Alloantisera Blocking Treatment of MLR Responder Cells. Viable normal spleen cells were incubated 1 h at 4°C at 3 × 10⁷/ml in NMS or antiserum diluted 1:10 in HBSS with 2% FCS. Treated cells were washed extensively in HBSS and added to MLR as responder cells. In blocking treatment of cells from 24 h MLR, cells were harvested and washed as above, divided into aliquots and incubated 1 h, 4°C at 2–3 × 10⁷/ml with NMS or antiserum diluted 1:10, which had been preadsorbed with cells of the strain used as stimulator cell in MLR. Cells were washed extensively in HBSS, and each treatment group was divided into three aliquots which were pulsed for 40 min at 4°C with culture medium with 2% FCS, or undiluted control or suppressor factors. Cells were again washed and replaced in culture wells containing retained washed adherent cells. Cultures were harvested 72 or 96 h after culture initiation.

Results

I-Region Determinants on MLR Suppressor T Cells. Molecules encoded by the I-C, but not I-J subregion, are active components of MLR-TsF (16). T suppressor (Ts) cells which secrete I-C antigen-containing suppressor molecules may also be expected to
display I-C determinants as cell surface constituents. We therefore have attempted to characterize the phenotype of MLR-Ts with regard to expression of I-C, as well as other I-region encoded antigens. Alloantigen-primed spleen cells were treated with various anti-I region sera and complement before the 24 h in vitro restimulation culture with cells of the allogeneic priming strain. Supernates of these cultured cell combinations were then assayed for suppressor activity in MLR with responder cells of a mouse strain identical to that used as the source of suppressor cells (Table 1).

Antiserum A.TH anti-A.TL (anti-J<sup>k</sup>) (group 1) and another serum, which possesses specificity for I-C<sup>d</sup> determinants, B10.S(9R) anti-B10.HTT (group 6), eliminated production of MLR-TsF by CBA (H-2<sup>k</sup>) splenocytes. Similarly, two antisera specific for I-C<sup>d</sup> products, (B10.A(4R) × B10.AM)F<sub>1</sub> anti-B10.A(2R) (group 8) and B10.HTT anti-B10.S(9R) (group 9), abrogated production of MLR-TsF by BALB/c (H-2<sup>d</sup>) suppressor cells. Antiserum B10.S(9R) anti-B10.HTT (anti-C<sup>d</sup>S<sup>d</sup>G<sup>d</sup>) and (B10.A(4R) × B10.AM)F<sub>1</sub> anti-B10.A(2R) (anti-C<sup>d</sup>S<sup>d</sup>G<sup>d</sup>) were both used in previous studies to identify I-C gene products in MLR-TsF (21). An observation of particular interest was that antiserum B10.A(3R) anti-B10.A(5R), specific for I-J<sup>4</sup> subregion products, also removed H-2<sup>k</sup> suppressor activity (group 3). We have previously established that B10.A(3R) anti-B10.A(5R) antiserum of the same pool, as well as other antisera with activity against I-J<sup>4</sup> gene products, do not adsorb suppressor molecules produced by alloantigen-primed cells of the H-2<sup>k</sup> haplotype (16). In the present study, three additional antisera, (A.BY × B10.HTT)F<sub>1</sub> anti-A.TL (group 2), (B10.D2 × A.BY)F<sub>1</sub> anti-B10.A(5R) (group 4), and B10.HTT anti-B10.S(9R) (group 5), which share anti-I-J<sup>4</sup> specificity, also eliminated suppressor activity from CBA MLR-Ts. In contrast,
antiserum (A.BY × B10.HTT)F1 anti-A.TL (anti-I-A^k, I-B^k, I-J^k) adsorbed with Con A-activated B10.A(5R) thymocytes (I-J^k) (group 2a), did not prevent MLR-TsF activity. Certain of the antisera which remove suppressor cell activity also potentially contain activity against H-2-associated Qa-1^a determinants. However, neither CBA nor BALB/c strains express this antigen.

In contrast, determinants encoded by the I-A and I-B subregions are not expressed on MLR suppressor cells. After adsorption with B10.A(5R) cells, the (A.BY × B10.HTT)F1 anti-A.TL antiserum retained significant cytotoxic activity against CBA spleen cells, presumably directed against I-A/B^k subregion determinants. Nevertheless, as previously noted, the adsorbed antiserum did not eliminate suppressor cell activity. In addition, treatment of A strain suppressor cells with antiserum (B10.S(9R) × A.TFR5)F1 anti-A.TL, which contains cytotoxic activity against I-A/B^k antigens, failed to affect subsequent suppression (group 7).

Collectively, these data suggest that I-J as well as I-C gene products are expressed by one or more subpopulations of primed cells critical to generation of MLR-TsF. The relevant I-C and/or I-J determinants exist on a subpopulation(s) of cells which do not display I-A and I-B subregion products.

**Dual Expression of I-C and I-J Subregion Determinants on MLR Suppressor T Cells.** Elimination of MLR suppressor activity by the removal of alloantigen-primed cells bearing either I-C or I-J encoded determinants, suggested either that gene products of the two subregions are expressed on the same MLR-Ts cell subpopulation, or that suppressive activity of I-C-bearing cells requires participation of a distinct set of cells bearing I-J subregion molecules. To differentiate between these alternatives, spleen cells from C57BL/6-sensitized CBA mice were divided into two aliquots; one was treated with antiserum B10.S(9R) anti-B10.HTT and complement to remove I-C^a-positive cells, whereas the other aliquot was exposed to B10.A(3R) anti-B10.A(5R) antiserum and complement to remove I-J^a-positive cells. Nonviable cells were removed, and the two preparations were mixed in equal parts and cultured with C57BL/6 stimulating cells for suppressor factor production (Table II). The cell mixture failed to reconstitute significant suppressor activity, suggesting that I-C and I-J gene products are expressed on a single population of cells critical to MLR suppression, and arguing against synergistic activity between discrete subpopulations of I-C-and I-J-positive cells.

**T-Cell Expression of I-C Determinants.** Immunoregulatory molecules encoded by the I-J subregion are limited to expression on a subpopulation of T lymphocytes (4, 21). Other I-region controlled molecules with regulatory properties are similarly represented selectively on T cells but not B cells (21). Because MLR-stimulating determinants associated with the I-C subregion are expressed only on T cells (22), it was of interest to determine if regulatory I-C molecules are restricted to T-cell expression as well. Antiserum (B10.A(4R) × B10.AM)F1 anti-B10.A(2R) (anti-C^aS^dG^e) was therefore adsorbed with BALB/c splenic B cells after anti-Thy-1.2 serum and complement treatment, nylon wool-nonadherent splenic T cells, or Con A-activated thymocytes. MLR-Ts were then treated with unadsorbed or adsorbed antisera plus complement, and suppressor factor activity was assessed in MLR with BALB/c responder cells (Fig. 1). Antiseras adsorbed with splenic B cells retained the capacity to remove MLR-Ts and eliminate MLR-TsF activity. In contrast, adsorption of the anti-I-C^a serum with either nylon wool-nonadherent T cells or Con A-activated thymocytes removed anti-
**Table II**  
*Dual Expression of I-C*<sup>+</sup> and *I-J*<sup>+</sup> Determinants on MLR-Ts*

| Group | Source | Antiserum treatment | CPM H<sup>3</sup>TdR<sup>*</sup> | Percent control MLR response |
|-------|--------|---------------------|-----------------------------|------------------------------|
| 1     | CBA    | NMS + C             | 3736 ± 285                  | 3121                         |
| 2     | CBA<sup>B6</sup> | NMS + C           | 2461 ± 139                  | 1618                         | 52                           |
| 3     | CBA<sup>B6</sup> | B10.S(9R) anti-B10.HTT + C (Anti-C<sup>A</sup>S<sup>G</sup>) | 3367 ± 437                  | 2746                         | 88                           |
| 4     | CBA<sup>B6</sup> | B10.A(3R) anti-B10.A(5R) + C (Anti-J<sup>+</sup>) | 3479 ± 198                  | 2899                         | 93                           |
| 5     | CBA<sup>B6</sup> | B10.S(9R) anti-B10.HTT + C | 3527 ± 422                  | 2855                         | 91                           |

* Mean cpm ± SEM of four replicate cultures. Data from an experiment representative of a total of four experiments with similar results.

† Control (CBA) and suppressor (CBA<sup>B6</sup>) cells were treated with NMS or alloantisera and complement before culture for factor production. Culture group 5 contained 1 × 10<sup>7</sup> cells from each antiserum-treatment group as indicated.

**I-C<sup>+</sup>** activity specific for MLR-Ts, thus allowing generation of MLR-TsF.

**I-Region Determinants on Targets of MLR-TsF.** It has been reported that antisera specific for I-J determinants on keyhole limpet hemocyanin-induced suppressor factor were also able to block or eliminate primed cells which act as targets of I-J<sup>+</sup> suppressor factor (23). These data have been interpreted to suggest that determinants of molecules encoded in the I-J subregion may be acceptor sites for the suppressive T-cell factor (21, 23). Thus, it was of interest to determine if sera detecting I-C determinants on MLR-TsF could similarly interfere with MLR-TsF interaction with unprimed target cells in MLR. CBA responder cells were incubated with antisera specific for I-C<sup>+</sup>, I-J<sup>+</sup>, and I-A/B<sup>+</sup> determinants in the absence of complement, before addition to assay MLR cultures with control and suppressor factors (Fig. 2A). None of the anti-I subregion sera inhibited MLR-TsF activity. In previous studies, responder cells activated by 24 h MLR culture were highly sensitive to brief MLR-TsF pulse, whereas nonactivated responder cells were unaffected by similar MLR-TsF exposure, suggesting the importance of activation in effective display of receptors for suppressor molecules (20). Thus, blocking studies were repeated with CBA responder cells which were harvested after 24 h culture with C57BL/6 or control CBA stimulator cells and exposed to the anti-I subregion sera (Fig. 2 B). Brief MLR-TsF exposure to previously activated responder cells generated profound suppression; preincubation of responder cells with antisera specific for I-C or other determinants failed to prevent MLR-TsF interaction with responder cells. Extension of the assay period for an additional 24 h provided essentially identical results. In addition, incubation of responder cells with antisera under other conditions of incubation period, incubation temperature, or antisera concentration were similarly ineffective.

Cytolytic removal of cells expressing I-region determinants from MLR responder cells was used as an alternate approach to determination of the possible role of I-C or other I-region molecules on the MLR-TsF target cell. Cytotoxic elimination of I-C-bearing cells from CBA or BALB/c responder cell preparations before MLR initiation failed to inhibit MLR-TsF suppression (Fig. 3 A). Depletion of I-A/B<sup>+</sup>-positive cells
resulted in a modest reduction of suppression. Because it has been reported that I-region antigen expression may be altered or increased with cell activation (24, 25), it is possible that relevant I-C-positive cells appear as effective targets only after activation. Thus, CBA or BALB/c responder cells harvested from 24 h culture with C57BL/6 or control syngeneic stimulator cells were treated with antisera specific for I-subregion-encoded determinants and complement before pulping with factor (Fig. 3B). The elimination of cells expressing I-C, I-J, or I-A/B antigens from an activated responder cell population did not reduce suppression resulting from MLR-TsF pulse. Moreover, proliferative responses of cultures treated with antisera and complement without factor pulse were unaltered in comparison to NMS controls, confirming previous observations that MLR responder cells are Ia negative (26), and suggesting that they retain an Ia-negative phenotype after MLR activation.

Discussion

The present studies were undertaken to establish the presence and participation of I-region-encoded molecules in regulatory interactions among T lymphocytes respond-
Fig. 2. Effect of anti-I subregion serum blocking treatment of MLR responder cells on subsequent MLR-TsF activity. Antisera (B10.S(9R) x A.TFR5)F1 anti-A.TL (anti-A^B^), (A.BY x B10.HTT)F1 anti-A.TL (anti-A^B^), B10.A(3R) anti-B10.A(3R) (anti-I^a^), and B10.S(9R) anti-B10.HTT (anti-I^c^S^G^) were used in the absence of complement, as described in Materials and Methods, to treat CBA responder cells before exposure to MLR-TsF. Panel A depicts MLR responses of responder cells treated with antisera before culture initiation, with MLR-TsF subsequently present for entire culture period. Panel B illustrates MLR responses of responder cells activated in MLR for 24 h before antiserum-blocking treatment, 40 min MLR-TsF pulse, and return to culture. Open bars indicate treatment with control factor; hatched bars indicate treatment with suppressor factor. Numbers over hatched bars are percent control MLR response. Data represent mean responses of two to three experiments performed with each antiserum.

ing to MHC antigens. We have demonstrated that alloantigen-primed suppressor cells display molecules encoded by the I-C subregion; antigenic determinants of the same subregion are also expressed by the secreted suppressor product, MLR-TsF (16). The I-C-bearing MLR-Ts cell is distinguished by concomitant expression of I-J
encoded determinants, thus relating the MLR suppressor T cell to antigen-specific suppressor systems. In contrast, no evidence was found for participation of responder cell I-C molecules in the interaction of MLR-TsF with its target cell. Neither blocking of cell surface I-C antigens nor cytotoxic elimination of I-C-bearing cells with anti-I-C sera inhibited MLR-TsF-mediated suppression. Thus, these findings may suggest a receptor-ligand recognitive interaction between suppressor molecules and a distinct receptor on the target cell, rather than a homology interaction between like (I-C) molecules.

I-J subregion determinants are expressed on a subpopulation of peripheral T cells which is characterized by suppressive function (4-9, 14). Similarly, I-J determinants
are found on certain antigen-specific suppressor factors (10-12), emphasizing functional specialization of this subregion and suggesting a direct role for I-J products in mediation of immune regulation. I-C gene-controlled regulation of MHC antigen-stimulated responses thus constitutes a genetically unique T-cell immunoregulatory system. It is particularly interesting that MLR suppressor cells display not only surface I-C determinants, but determinants of the I-J subregion as well. Suppressive effector activity of soluble factors secreted by these cells however is associated solely with I-C molecules (16). Thus, the I-J gene product is expressed as a cell surface functional marker in the apparent absence of subsequent participation in MLR-TsF immunoregulation. Because I-J determinants are general concomitants of antigen-specific suppressor activity, they may in fact serve a direct function in initial antigen interaction with or activation of any antigen-driven suppressor cell. Alternatively, I-J molecules identified on MLR-Ts may be passively acquired from a distinct I-J positive cell, and act to secondarily stimulate I-C bearing suppressor cells. Allogeneic MHC antigens are passively displayed by activated T lymphocytes (27); acquisition of syngeneic Ia molecules from other activated T cells or macrophages is a possibility which can not presently be discounted. However, if both I-C and I-J antigens are products of the same cell, both types of molecules are probably shed or secreted by activated MLR-Ts cells. I-C-restricted suppression might then be imposed not by sole availability of I-C-bearing suppressor molecules but rather by restricted expression of I-C-specific receptors on a subpopulation of cells activated by allogeneic I-region antigens in MLR.

I-C molecules with immunoregulatory properties are not expressed on B cells, but are clearly identified on a subpopulation of T cells, and may show enhanced display with T-cell activation. Comparison of the adsorbing properties of normal nylon wool-nonadherent spleen T cells and Con A-activated thymocytes in a preliminary adsorbing cell dose analysis, suggests that Con A-activated thymocytes have approximately twice the adsorptive capacity of resting spleen T cells (S. Rich, unpublished observation). I-C determinants have been reported in addition on a subpopulation of splenic macrophages (28); the relationship of macrophage I-C molecules to those which characterize MLR-Ts is unknown.

MLR-stimulating determinants (22), specificity Ia. 6 (29), and control of responsiveness to sites 1 and 2 of sperm whale myoglobin (30), have also been mapped to the I-C subregion. The relationship of genes controlling these traits is unknown. Some of the same antiserum pools used to characterize I-C-controlled MLR-stimulating determinants were also used to identify I-C gene products on MLR-Ts. In addition, data of Okuda and David (22) are consistent with existence of a minor population of cells expressing I-C^k MLR-stimulating determinants which is also removed by anti-I-J^k sera and complement. These data could again reflect dual I-C and I-J determinant expression, and similarity of I-C determinants on MLR-Ts and I-C-encoded MLR-stimulating molecules.

Demonstration of I-region molecules as essential components of soluble regulatory factors (10-12, 16) suggests direct functional involvement of these molecules in mediation of regulatory activity. Possible mechanisms of factor-target cell interaction include like-like molecular association of identical structures, as well as receptor-ligand interactions of distinct complementary structures. In these studies, results of both blocking and cytotoxic analysis with anti-I-C sera failed to support a direct role
of serologically detected I-C or other I-region molecules of the responder cell in interaction with MLR-TsF at the target cell surface. Lack of serologic effect may possibly be ascribed to quantitative effects of relevant Ia antigen display. Target cells may express I-C or other determinants in particularly low concentration, even after MLR activation, resulting in resistance to standard antibody and complement-mediated lysis. However, another population of alloantigen-activated T cells, MLR T suppressor cells, was effectively eliminated by these antisera under identical conditions. Significant quantitative, if not qualitative differences in receptor display by primed cells, as well as the possibility of anti-I subregion sera possessing multiple antibody species which may also detect determinants of receptor structures, may play a role in differing results, such as those reported by Tada (21, 23). Preliminary observations have been reported by Germain et al. (14) in a two-cell suppressor pathway mediated by I-J-bearing suppressor factor stimulated by L-glutamic acid\textsuperscript{50}, L-tyrosine\textsuperscript{50} (GT-TsF). Cytotoxic anti-I-J antiserum treatment did not inhibit GT-TsF stimulated generation of secondary Ts from a nonprimed splenic cell population, although induced Ts ultimately expressed I-J determinants. Similarly, I-A-restricted induction of helper T-cell activity by an I-A-bearing macrophage factor was not inhibited by blocking treatment of target T cells by anti-I-A sera (31).

Recent evidence indicates that T cells recognize self-MHC gene products through specific cell surface receptors (32–34). Thus, it is possible that I-C molecules in MLR-TsF interact with target cells by virtue of anti-I-C receptors in a receptor-ligand fashion. Use of target cells from appropriately contrived bone marrow chimeras, which would allow manipulation of T-cell receptor specificity for "self" I-region determinants, as well as expression of I-region molecules, may permit further insight into the molecular basis of target cell interactions with immunoregulatory molecules.

**Summary**

The phenotypic expression of I-region determinants on cells producing and responding to MLR suppressor factor (MLR-TsF) was established in these studies. Alloantigen-activated MLR suppressor T cells (MLR-Ts), which produce MLR-TsF bearing gene products of the I-C subregion, were exposed to anti-I subregion sera and complement (C) before in vitro culture for MLR-TsF production. Suppressor activity was prevented by removal of cells bearing I-C determinants, whereas elimination of cells expressing I-A/B determinants had no effect. Interestingly, cytotoxic elimination of cells displaying I-J determinants also prevented MLR-TsF production. Admixture of anti-I-J and anti-I-C antiserum-treated cells for MLR-TsF production failed to reconstitute suppressor activity, indicating that I-C and I-J gene products are expressed on a single population of cells critical to MLR suppression, rather than on distinct interacting subpopulations. Anti-I-C serum activity specific for I-C\textsuperscript{+} MLR-Ts was removed by adsorption with nylon wool-nonadherent splenic T cells and concanavalin A-activated thymocytes; adsorption with splenic B cells from anti-Thy-1.2 serum and C-treated spleen failed to remove relevant anti-I-C activity. These data suggest that regulatory I-C molecules, like I-J molecules, are preferentially expressed on T lymphocytes. Expression of I-C, or other I-region molecules on responder cell targets of MLR-TsF activity was also investigated. Responder cells were pretreated with anti-I subregion-specific sera in blocking or complement-dependent cytotoxic protocols.
before addition to MLR with MLR-TsF. Neither blocking nor the cytotoxic removal of cells bearing I-C or other I-region determinants from MLR responder populations interfered with MLR-TsF suppression. Because it has previously been demonstrated that MLR-TsF interacts optimally with activated, I-C syngeneic target cells, blocking and cytotoxic studies with anti-I subregion sera were also performed with responder cells activated by 24 h culture in MLR in the absence of MLR-TsF. Brief MLR-TsF pulse after antiserum treatment generated marked suppression regardless of blocking or absence of cells bearing serologically detected I-region determinants. I-C restricted suppression may thus be mediated not by interaction with I-C-bearing cells, but by target cells which exist in requisite association with populations of I-C cells.

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