REGULATORY MECHANISMS IN CELL-MEDIATED IMMUNE RESPONSES

IV. Expression of a Receptor for Mixed Lymphocyte Reaction Suppressor Factor on Activated T Lymphocytes*

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Proliferative responses in mixed lymphocyte reactions (MLR)[1] are suppressed by a soluble factor released by alloantigen-activated splenic T cells (1, 2). We have established that such suppressive T-cell factors derived from one strain only suppress responses of strains histocompatible for regions of the *H-2* complex between *I-E* and *D* (1, 2). *H-2*-dissimilar MLR responder cells are unaffected by active suppressor factors. The data suggest that a receptor specific for that factor and required for an active suppressive interaction is expressed by genetically homologous cells. *H-2*-dissimilar responder cells would lack the appropriate genetically determined receptor and upon exposure to suppressor factors would be unaffected. A similar "acceptor" site for primed helper T-cell factor has been postulated on B cells (3). Genes mapped in the *I* region control functional expression of the acceptor, as well as interacting factor molecules. Thus it may be through association of such molecules, either released or on cell membranes, with B-cell surface structures that appropriate cell interactions in antibody synthesis are achieved.

Accordingly we have tested the ability of cells, used as MLR responder cells, to adsorb suppressor factor activity as an indication of a receptor for MLR suppressor molecules. The results suggest that such a receptor structure is dynamically expressed by a subpopulation of T lymphocytes only after a triggering antigenic or mitogenic signal. The receptor is not present or perhaps not functional on resting cells. *H-2* control of the receptor molecule is suggested by the inability of activated *H-2*-dissimilar T cells to interact with and remove suppressor activity.

Materials and Methods

Mice. BALB/c and DBA/2 mice were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, Texas. C57BL/6 mice were obtained from TIMCO Breeding Labora-

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction(s); PHA, phytohemagglutinin.

tory Inc., Houston, Texas. C3H/He and A/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Experiments were performed with 6- to 14-wk-old male animals.

Concanavalin A. Twice recrystallized concanavalin A (Con A) (ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio) was dissolved in Hanks' balanced salt solution (HBSS) (Microbiological Associates, Bethesda, Md.) at 1 mg/ml, stored at 4°C for not more than 1 wk, and diluted to desired concentration immediately before use.

MLR. MLR were prepared as previously described (4), with the exception of the culture medium employed. Briefly, responder and stimulator cell populations were cultured in equal numbers, 1×10^6 cells of each, in 0.2 ml cultures in supplemented Eagle's minimal essential medium (MEM) (5, 6) with 10% fetal calf serum (FCS) (Reheis Chemical Co., Kankakee, Ill) and 50 μg/ml gentamicin (Schering Corp., Kenilworth, N. J.). Stimulator cells (designated throughout by subscript m) were treated before addition to MLR with mitomycin C (Sigma Chemical Co., St. Louis, Mo.). MLR cultures were incubated in an atmosphere of 10% CO_2, 7% O_2, and 83% N_2 at 37°C. DNA synthesis in MLR was assayed by adding 1.0 μCi of tritiated thymidine (sp act 2.0 Ci/mmol; New England Nuclear, Boston, Mass.) to cultures for the final 18 h of a 72 h incubation period. Exceptions to this protocol are subsequently detailed.

Data from separate experiments are expressed as mean counts per minute of four to six 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). E-C from grouped replicate experiments represent mean E-C from three to five experiments. Percent MLR response was calculated according to the following formula:

$$\frac{\text{(E-C) of MLR with supernate}}{\text{(E-C) of MLR without supernate}} \times 100 = \% \text{ control MLR response.}$$

Data were analyzed statistically by the Student's *t* test.

Preparation of Suppressor and Control Supernates. Suppressor supernates were produced as previously described (1). Briefly, normal mice were injected into hind foot pads with 2×10^7 allogeneic spleen cells. 4 days later alloantigen-activated spleen cells were 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 spleen cells with equal numbers of mitomycin C-treated syngeneic cells.

Preparation of Adsorbing Cells. Cellular adsorbants were prepared from fresh normal thymocytes and spleen cells, and from thymocytes or spleen cells activated in vitro with mitogen or allogeneic cells. Purified phytohemagglutinin (PHA) was obtained from Burroughs Wellcome & Co., Triangle Park, N. C.; lipopolysaccharide W (LPS) from *Escherichia coli* 0127:B8 was purchased from Difco Laboratories, Detroit, Mich. Mitogen-stimulated cells were prepared by incubating spleen or thymus cells with Con A (3 μg/ml), PHA (1 μg/ml), or LPS (100 μg/ml) at 10^7 cells/ml in supplemented MEM containing 5% FCS under Mishell-Dutton conditions (5) for 48 h.

Alloantigen-stimulated adsorbing cells were prepared by co-culture of normal spleen cells and allogeneic or syngeneic mitomycin C-treated spleen cells at 10^7 cells/ml final concentration of each population under conditions as described above. Normal unstimulated thymocytes or spleen cells were prepared as single cell suspensions and washed extensively. At the time of adsorption, cultured cells were harvested and washed four times in HBSS or, in the case of Con A-activated cells, with 0.15 M methyl-α-D-mannoside in HBSS.

Adsorption of Supernates. Suppressor and control supernates were incubated with $2.5\text{–}3.0 \times 10^8$ packed prepared adsorbing cells/ml fluid at 4°C for 30 min with frequent mixing. Thereafter the cells were removed by centrifugation.

Results

Removal of Suppressor Factor Activity by Activated Thymocytes. Since normal splenic lymphocytes are used as responder cells for MLR, these were used in initial attempts to adsorb suppressor activity (Fig. 1). As previously described (1), supernates of cultured alloantigen-activated BALB/c spleen cells

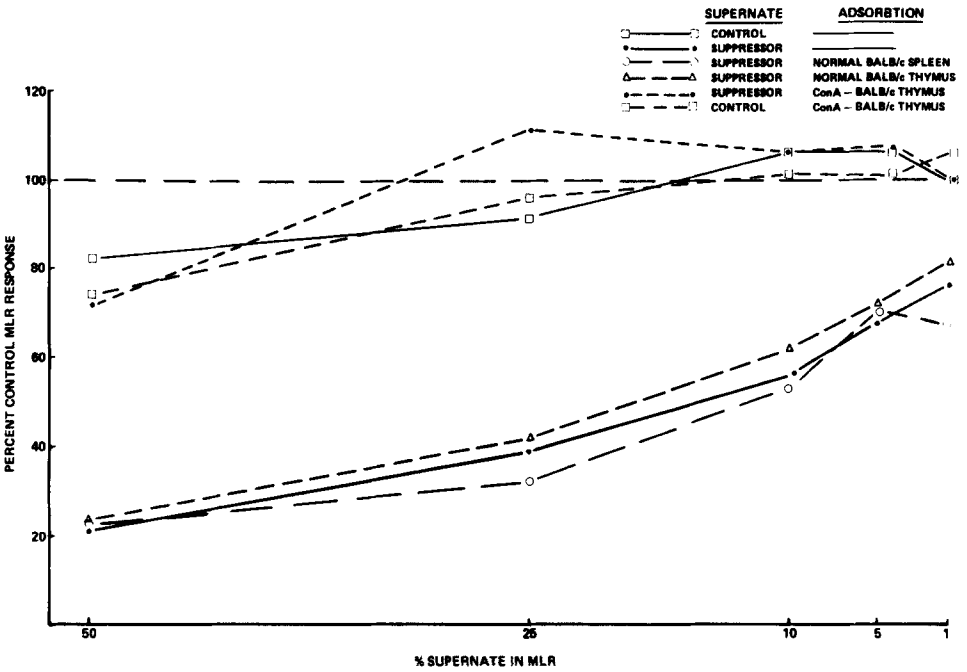

FIG. 1. Activity of MLR suppressor factor after exposure to normal or activated lymphocyte preparations. Supernates were derived from normal (control) or C57BL/6-activated (suppressor) BALB/c spleen cells, and adsorbed with the lymphocyte preparations indicated. Activity was tested in MLR of BALB/c responder and BALB/c or C57BL/6 stimulator cells. Data represent mean responses of 4–12 experiments.

strongly suppressed MLR responses in a dose-dependent fashion. Incubation of suppressor factor with normal BALB/c spleen cells or thymocytes before addition to MLR had no effect on its suppressive activity. Since indirect evidence exists that T-cell-mediated suppression affects primarily the proliferative phase of the response rather than blocking initial antigen recognition (1, 7), the possibility was then investigated that the receptor for suppressor factor is expressed only after antigenic triggering. As a model, thymocytes activated by Con A were tested for their ability to remove or inactivate suppressor activity. In contrast to the lack of effect of normal thymocytes, suppressor activity was significantly reduced by exposure to Con A-activated thymocytes. Inhibition of MLR occurred only at the highest concentration of activated thymocyte-adsorbed suppressor factor. It is important to note that control factor, similarly incubated with Con A-activated thymocytes had no effect, enhancing or otherwise, on MLR responses.

Adsorbing Capacity of Various Activated Lymphocyte Populations. The subpopulation of cells which is able to interact with suppressor factor was characterized by studying the adsorbing capacity of various lymphocyte populations activated by several mitogens (Fig. 2). While thymocytes stimulated with Con A effectively removed suppressor activity, thymocytes stimulated by another T-cell mitogen, PHA, showed no adsorbing capacity. Surprisingly, Con A-activated spleen cells were also ineffective adsorbants. In addition, an activated

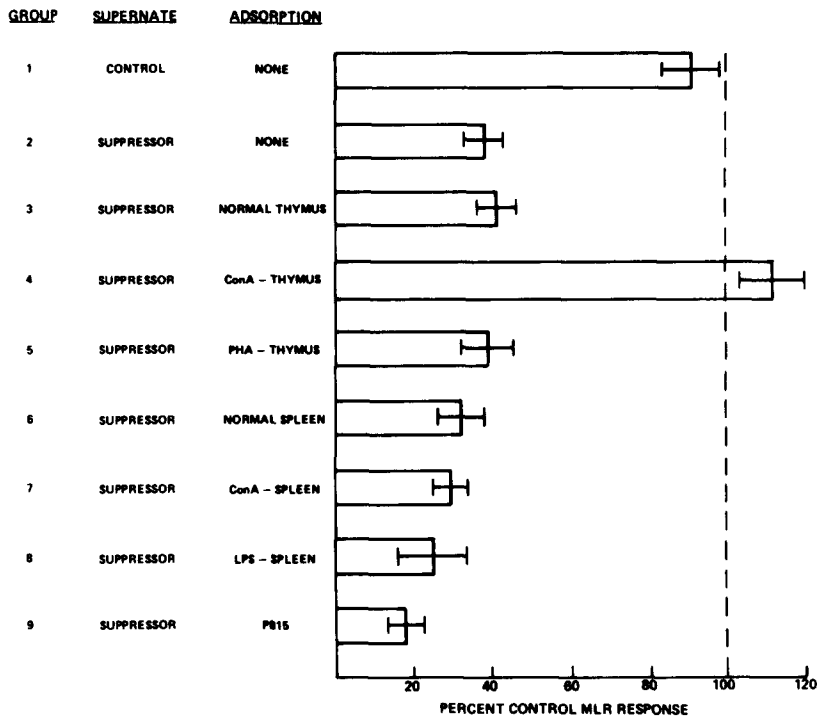

FIG. 2. Activity of MLR suppressor factor after adsorption with various normal or mitogen-activated lymphocyte preparations. Supernates from C57BL/6-activated (suppressor) or normal (control) BALB/c spleen cells were adsorbed with the lymphocyte preparations indicated. Activity was tested in MLR of BALB/c responder and BALB/c or C57BL/6 stimulator cells (final concentration in MLR, 25%). Data represent mean responses ± SEM of 2–12 experiments.

B-cell population derived from LPS-stimulated spleen cells did not affect MLR suppressor activity. All of the described adsorbing cell preparations possessed 77–94% blast cell forms. Also tested for suppressor factor adsorption was a neoplastic cell line of the same *H-2* haplotype as both the cells producing suppressor factor and the Con A-activated thymocytes which adsorbed factor activity. Incubation of factor with P815 mastocytoma cells resulted in no reduction of suppressor activity; in fact, slight enhancement of suppression was observed.

Genetic Restriction of Suppressor Factor Adsorption. As described previously, factor which suppressed syngeneic MLR responder cells had no effect on cells which did not possess the appropriate *H-2*-region homology (1, 2). Consequently, it was of interest to test *H-2*-incompatible Con A-activated thymocytes with regard to their ability to interact with suppressor molecules. Suppressor factor produced by alloantigen-activated BALB/c ($H\text{-}2^d$) spleen cells was incubated with normal or Con A-activated thymocytes of various *H-2* haplotypes and tested in MLR with BALB/c responder cells (Fig. 3).

Normal thymocytes of all strains utilized showed little ability to remove suppressor activity. Again, activated BALB/c thymocytes effectively removed suppressor activity. *H-2*-identical DBA/2 ($H\text{-}2^d$) Con A-activated thymocytes

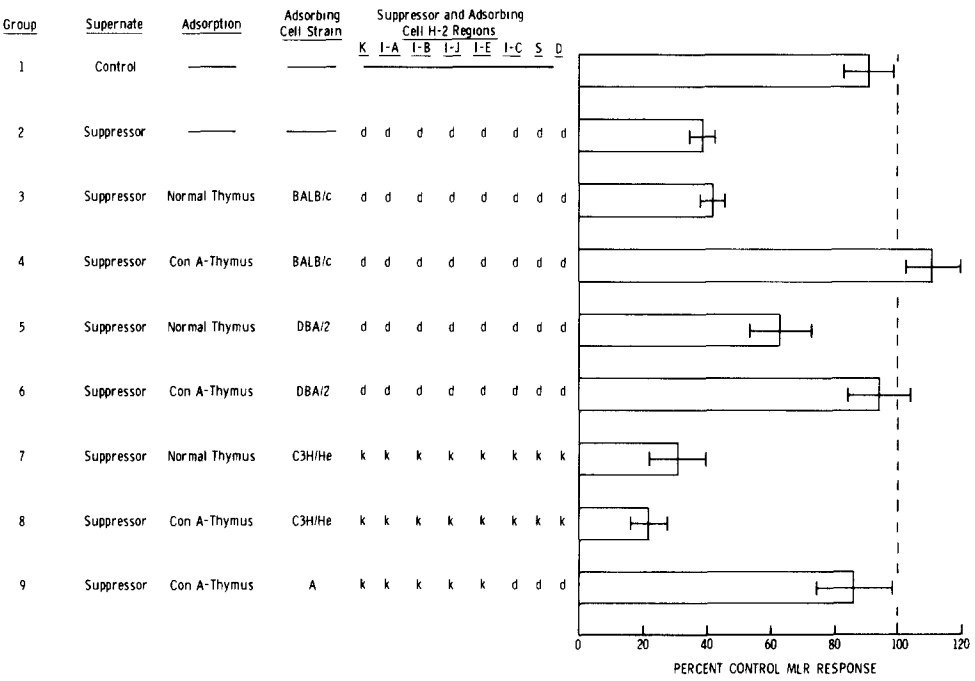

FIG. 3. Activity of suppressor factor after adsorption with normal or Con A-activated thymocytes from various mouse strains. Supernates from C57BL/6-activated BALB/c spleen cells (suppressor) were adsorbed with lymphocyte preparations as indicated and tested in MLR of BALB/c responder and BALB/c or C57BL/6 stimulator cells (final concentration in MLR, 20%). Data represent mean responses ± SEM of 3–12 experiments.

showed similar adsorbing capacity. In contrast, suppressor activity remained intact after exposure to *H-2*-dissimilar activated C3H/He ($H\text{-}2^k$) thymocytes. Finally, BALB/c suppressor factor was adsorbed by activated thymocytes of strain A, which share *H-2* regions to the right of *I-E* with the $H\text{-}2^d$ haplotype of BALB/c.

Supernate-target cell interactions showed similar genetic restrictions when the suppressor factor tested was produced by cells of another *H-2* haplotype, C3H/He ($H\text{-}2^k$) (Fig. 4). Again, Con A-activated C3H/He thymocytes, but not normal or Con A-activated C3H/He spleen cells, adsorbed suppressor factor. Normal syngeneic thymocytes also partially abrogated suppressor activity. Neither normal nor Con A-activated *H-2*-dissimilar BALB/c ($H\text{-}2^d$) thymocytes were effective adsorbants.

Suppressor Factor Adsorption by Alloantigen-Activated Lymphocytes. Since these adsorption studies were initially predicated on the effects of suppressor factor on cells which were responding to alloantigens in the context of the MLR, it was pertinent to use, as adsorbing cell populations, spleen cells which were stimulated by alloantigen rather than by mitogens. BALB/c or C3H/He spleen cells were incubated for 48 h with syngeneic or allogeneic mitomycin C-treated cells; they were harvested, washed extensively, and prepared as adsorbing cells for BALB/c factors (Fig. 5). As a control for the effects of alloantigen-induced activation, BALB/c and mitomycin C-treated C57BL/6 ($H\text{-}2^b$) cells were pre-

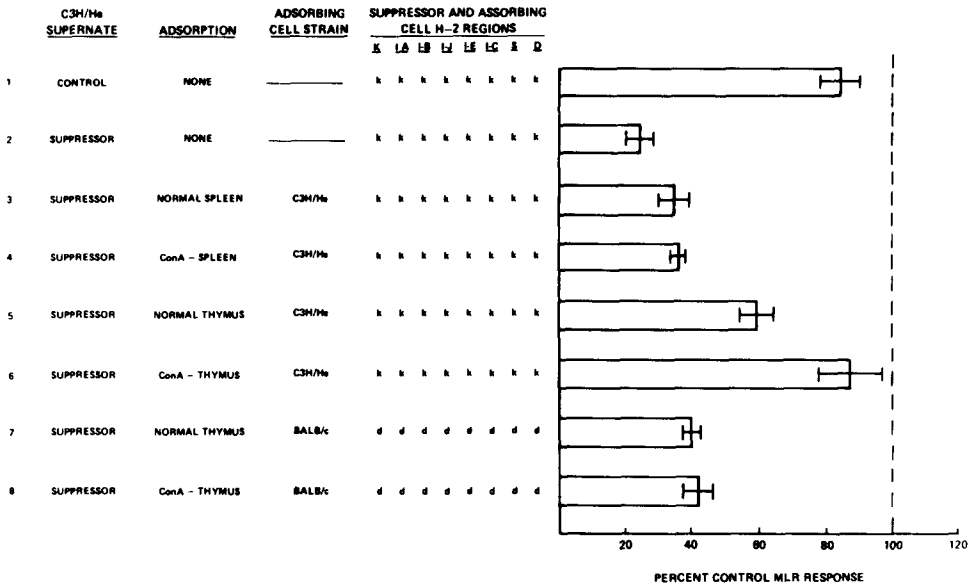

FIG. 4. Activity of C3H/He suppressor factor after adsorption with normal or Con A-activated lymphocytes from various mouse strains. Supernates from C57BL/6-activated C3H/He spleen cells (suppressor) were adsorbed with lymphocyte preparations as indicated and tested in MLR of C3H/He or C57BL/6 stimulator cells (final concentration in MLR, 20%). Data represent mean responses ± SEM of three experiments.

pared and combined only at the time of adsorption. BALB/c spleen cells cultured with syngeneic cells showed no adsorbing effect. In contrast, BALB/c cells stimulated by culture with C57BL/6 cells significantly removed suppressor activity. The same cell pair combined only at the time of factor adsorption was inactive. Finally, C3H/He ($H\text{-}2^k$) spleen cells also stimulated by C57BL/6 cells did not reduce BALB/c ($H\text{-}2^d$) suppressor activity. The C57BL/6 target cell in the various adsorbing mixtures was not a primary participant, as demonstrated by the absence of suppressor activity adsorption in groups 6 and 7.

Effect of Suppressor Factor Adsorption on Subsequently Cultured MLR Responder Cells. Although suppressor activity was clearly unaffected by exposure to normal thymocytes or spleen cells, it nevertheless was possible that normal cells functionally bind suppressor molecules and that the strength of remaining suppressor activity masks a relatively slight reduction of suppressor molecule concentration. Thus MLR responder cells, either alone or in combination with syngeneic or allogeneic stimulator cells were preincubated with control or suppressor factors under adsorbing conditions, washed, and cultured in MLR (Fig. 6). MLR cultures were assayed at 72, 96, and 120 h after culture initiation. One set of cultures was prepared in the usual fashion with control and suppressor factors present throughout the entire culture period to serve as an assay of suppressor activity in the supernates utilized. Preincubation (0 h adsorption) of responder cells alone (not shown) or in combination with stimulator cells had no significant effect on proliferative responses of these cells. Incubation of responder cells at 0 h with suppressor or control factors at 37°C rather than 4°C similarly had no effect (data not shown).

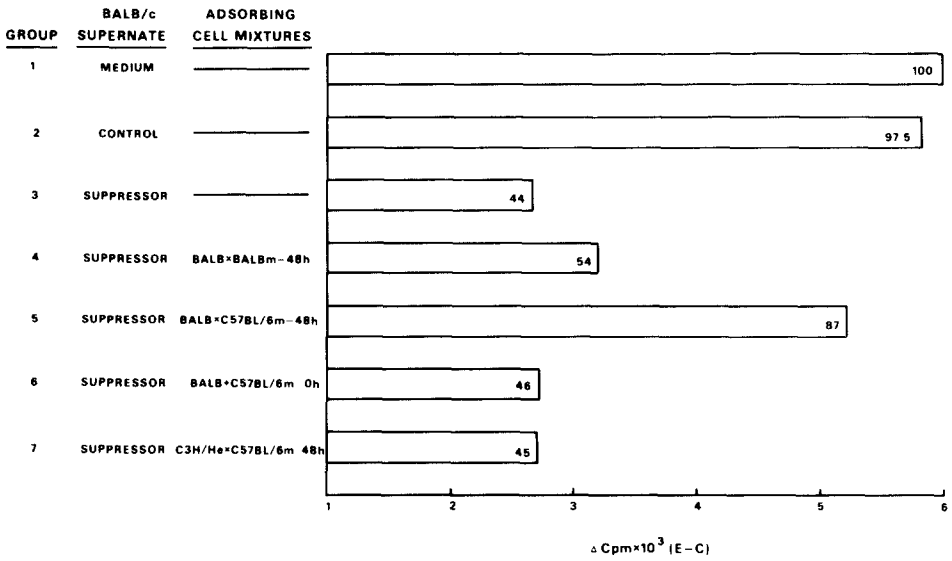

FIG. 5. Suppressor factor activity after adsorption with alloantigen-activated lymphocytes. Supernates from C57BL/6-activated BALB/c spleen cells (suppressor) were adsorbed with syngeneic or allogeneic cell mixtures which had been cultured for 48 h or prepared and combined at the time of adsorption (0 h). The supernates were tested in MLR of BALB/c responder and BALB/c or C57BL/6 stimulator cells. Data represent mean E-C counts per minute of three experiments. Figure in each bar represents percent control MLR response.

Since it appeared that normal cells did not functionally bind suppressor factor, it was then of interest to determine the point in MLR culture at which such binding might be identified. At various times after culture initiation MLR were harvested, exposed to suppressor and control factors under adsorbing conditions, washed, and returned to culture. MLR cultures exposed to suppressor factor for 40 min 4 h after culture initiation showed significantly ($P < 0.005$) reduced proliferation at all assay periods in contrast to cultures incubated with either control factor or medium. Cells similarly treated after 2 h in MLR showed equivocal results (not shown). Exposure of MLR cultures to suppressor factor for 40 min at 24 h produced inhibition which was quantitatively similar but delayed in comparison to that expressed after treatment at 4 h.

Discussion

Suppression of MLR requires genetic homology between alloantigen-activated suppressor T cells, from which a suppressive factor is derived, and the MLR responder cell (1, 2). Therefore we have postulated that a receptor specific for suppressor molecules is expressed by appropriately homologous responder cells and is lacking on *H-2*-dissimilar cells. We have attempted to identify such a receptor through adsorption or inactivation of suppressor factor activity by exposure to various normal and activated lymphocyte populations. The present results suggest that only after activation by mitogens or alloantigen are requisite receptors expressed by a subpopulation(s) of T lymphocytes which allow interaction with MLR suppressor molecules. Restriction of factor-target interac-

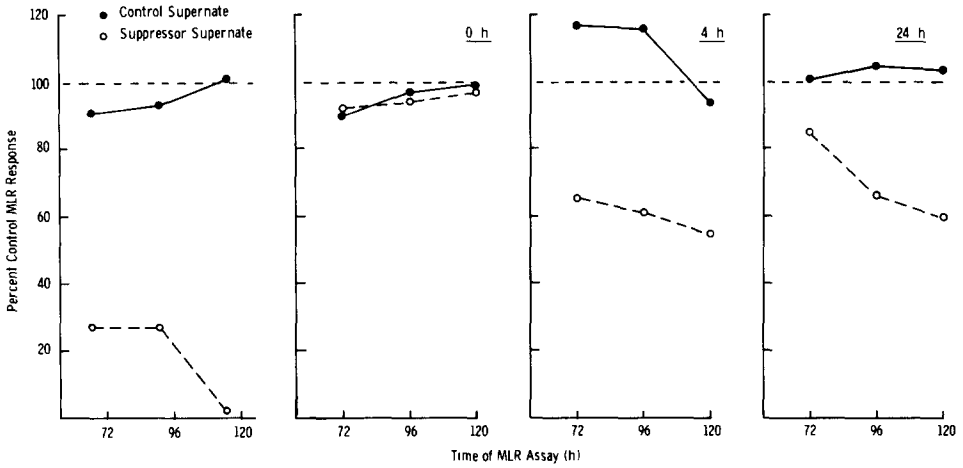

FIG. 6. Effect of suppressor factor adsorption on subsequently cultured MLR responder cells. Supernates present for entire MLR culture period (final concentration in MLR, 20% [left panel]) or incubated for 40 min at 4°C with MLR cells harvested at 0, 4, or 24 h after culture initiation. Thereafter the MLR cells were washed and returned to culture. MLR responses were assayed at 72, 96, or 120 h of incubation. Supernates from C57BL/6-activated (suppressor) or normal (control) BALB/c spleen cells were tested in MLR of BALB/c responder and BALB/c or C57BL/6 stimulator cells. Data represent mean responses of three experiments.

tion only under conditions of genetic identities in the *H-2* complex to the right of *I-E* is consistent with *H-2* control of receptor display.

The structure allowing interaction of responding cells with suppressor molecules is not present or perhaps not functional on resting, potentially alloantigen-reactive lymphocytes. Repeated experiments confirmed the observation of minimal or no effect on suppressor activity after exposure to normal freshly prepared or cultured, unstimulated thymocytes or spleen cells. Furthermore, under modified conditions of adsorption with unstimulated cells, using three- to fourfold greater concentration of adsorbing cells or various temperatures of incubation, suppressor adsorption did not occur (unpublished observations). Interaction with suppressor molecules, identified by loss of activity after exposure to target populations, occurred only when target cells had been first stimulated by alloantigen or mitogen.

Although a variety of mitogens were tested as stimulating agents with different lymphoid populations, only Con A-activated thymocytes were active adsorbants. It was important to determine if residual membrane-bound Con A was directly involved in suppressor factor inactivation, since this and other lectins are known to bind major histocompatibility complex gene products (8). This possibility was considered unlikely, however, since other cellular adsorbants prepared with Con A, either spleen cells or thymocytes histoincompatible to the factor-producing strain, did not affect suppressor activity. In addition, histocompatible lymphocytes activated by allogeneic cells showed analogous suppressor factor inactivation.

It was surprising that Con A-activated spleen cells did not adsorb factor activity since MLR responder cells are prepared from spleen cells and are

affected by suppressor factors. However, Con A-activated peripheral T cells have been reported to have a significantly lower density of certain cell surface antigens per cell (9) than Con A-activated thymocytes (10). A similar quantitative difference in display of the structure relevant to interaction with suppressor factor may also be identified in this system.

The mitogen studies suggest the subpopulation of immunocompetent cells which serves as the suppressor factor target. In MLR between heterogeneous populations of responder and stimulator cells, B cells may participate in a secondary fashion in response to signals generated by T-cell factors (11, 12). In previous studies (1, 2) using unfractionated responder and stimulator cell preparations, suppression of responding B cells, as well as T cells was possible. However, the LPS-stimulated, B-cell-enriched adsorbing population was completely ineffective in suppressor factor adsorption, thus suggesting that B cells are not the primary target of suppressor factor activity. In contrast, suppressor factor does interact with T cells, more specifically a particular subset of T cells. Thymocytes stimulated by the T-cell mitogen PHA, perhaps a more mature subset of thymocytes (13), showed no adsorptive capacity. Thus the ability to express structures capable of interacting with suppressor molecules appears to reside in that subpopulation of T lymphocytes which is characterized by Con A responsiveness. In the context of this demonstration of T-cell heterogeneity, it is interesting that PHA responsiveness and proliferation in MLR are also properties of distinct T-cell subsets (14).

Studies with alloantigen-stimulated adsorbing cells are pertinent to investigation of suppressor interaction with responding cells in MLR. Again stimulation of histocompatible cells, in this instance through alloantigen presentation, was required before inhibition of suppressor activity could be observed. Furthermore these studies demonstrated that loss of suppressor activity subsequent to exposure to certain activated cell preparations reflected functional binding of T-cell suppressor molecules to receptor structures on responding cells. Thus, MLR responder cells incubated with suppressor factor before culture initiation were not suppressed. However, after a short period of culture with allogeneic stimulating cells in MLR, responding cells become susceptible to the inhibitory effects of MLR suppressor factor. Nonsynchronous events of responder cell sensitization and perhaps of periodic display of suppressor receptor during the cell cycle may contribute to the moderate degree of suppression observed after a single brief exposure to suppressor factor.

In addition to an activating event, genetic compatibility between the suppressor factor-producing cell and the target cell was required for successful interaction. Thus, suppressor factor produced by alloantigen-activated BALB/c spleen cells ($H\text{-}2^d$) was adsorbed not only by Con A-activated BALB/c thymocytes but also by *H-2*-identical Con A-activated DBA/2 thymocytes. In contrast, the same factor retained its full suppressive activity after exposure to Con A-activated C3H/He thymocytes ($H\text{-}2^k$). Similar results were obtained when adsorbing cells were activated with alloantigen rather than mitogen.

Suppressor activity was also adsorbed from BALB/c factor exposed to activated A strain thymocytes ($H\text{-}2^a$), which share the *I-C*, *S*, and *D* regions with BALB/c. Failure of C3H/He cells to remove suppressor activity did not reflect

inability to interact with suppressor molecules since those target cells could adsorb suppressor activity of a factor produced by genetically identical *H-2*^{k} spleen cells. Thus identity for the regions to the right of *I-E* in the *H-2* complex was sufficient for interaction with and removal of suppressor molecules. Consequently these data indicate that the receptor structure for MLR suppressor molecules is controlled by a gene(s) in the right-hand regions of the *H-2* complex, probably within the *I* region. These observations are consistent with our previous demonstration of *I-C*-subregion control of suppressor cell-responder cell interaction in MLR (2).

The studies presented here suggest that after alloantigenic or mitogenic stimulus, T lymphocytes express a receptor, either through *de novo* synthesis or alteration of an existing structure, which then provides the appropriate site for interaction with suppressor molecules. An alternative but less likely mechanism of receptor display might be through passive acquisition by the activated T cell of molecules liberated by other cells. T cells activated in MLR bind both *K*- and *I*-region alloantigenic products of stimulator cells (15), as well as immunoglobulin (15, 16). However, unless it is postulated that the adsorbing capacities of mitogen and alloantigen-activated cells derive coincidentally from two entirely different mechanisms, the results of adsorption by Con A-activated thymocytes would be difficult to reconcile with the notion of receptor molecules passively acquired from a stimulator population. Similarly, genetic restrictions on factor adsorption by different alloantigen-activated adsorbing cells using the same stimulator cell strain are inconsistent with this postulate.

Both alloantigen and Con A activation of T lymphocytes induce or enhance the expression by these cells of Fc receptors (17–20) and Ia alloantigens (10, 20). Lack of B-cell adsorption, as well as genetic restriction of suppressor-target interaction suggest that Fc receptors are not a primary component of the suppressor target structure. Present observations are, however, consistent with the possibility that Ia molecules may be part of the receptor. Ia antigens are not expressed on PHA-stimulated lymphocytes (21) nor on P815 mastocytoma cells (22) and these populations do not adsorb suppressor molecules. Since Ia specificities are identified on normal (23) and mitogen-stimulated (9, 10, 21) B cells, it would be necessary to suggest a T-cell-restricted Ia expression, such as has been demonstrated for certain stimulator T cells in MLR (24). *I*-region gene control of other acceptor structures critical to regulatory T-cell interactions has been demonstrated (3, 25). Although MLR responders have been described as functionally Ia negative by the criterion of anti-Ia serum-mediated cytotoxicity (26), it is possible that Ia is present but not defined under these conditions. Alternatively, MLR responder T cells may require a triggering signal before they exhibit full expression of Ia specificities.

Determination of the functional character of the subset of T cells which bear the MLR suppressor receptor will be of great interest. Although the issue of the suppressor target is controversial, the work of Taniguchi and co-workers (27) indicates that it is the helper T cell which is directly affected by suppressor molecules, with consequent inhibition of antibody synthesis. Gershon similarly suggests the requirement for some helper activity to be present in order for suppression to be manifest (28). Since it has been demonstrated that Con A-

responsive cells encompass both helper and suppressor functional subsets, distinguishable by Ly expression (29), the Con A-activated adsorbing cell may represent a subpopulation of T cells with the capacity to enhance MLR proliferative responses. Such a functional T-cell subset, found predominantly in the thymus, has been suggested (30).

Cell surface modulation has gained critical attention as a central phenomenon in cellular events of division, movement, and interaction in several model systems (31). Regulation of immunocompetent cell social behavior through dynamic cell membrane events under major histocompatibility gene complex control, as postulated in this report, may have a role in developing theories of cell interactions in systems extending beyond those of immunological function.

Summary

Suppression of the mixed lymphocyte reaction (MLR) by a soluble factor produced by alloantigen-activated spleen cells requires genetic homology between the factor-producing cells and responder cells in MLR. The ability of lymphocytes used as MLR responder cells to adsorb MLR suppressor factor was tested to investigate the expression of a receptor structure for suppressor molecules. Normal spleen or thymus cells had no effect on suppressor activity. Concanavalin A (Con A)-activated thymocytes, however, effectively removed suppressor activity, suggesting that the receptor is expressed only after activation and is not present or not functional on resting cells. Significantly neither phytohemagglutinin- nor lipopolysaccharide-activated lymphoid cells adsorbed the factor. Furthermore, only Con A-activated thymocytes demonstrating genetic homology with the cell producing suppressor factor for *H-2* regions to the right of *I-E* were effective adsorbants. Alloantigen-stimulated spleen cells syngeneic to the suppressor cell also removed suppressor activity. These data support an hypothesis that subsequent to stimulation in MLR, T lymphocytes express a receptor, either through synthesis or alteration of an existing molecular structure, which then provides the appropriate site for interaction with suppressor molecules.

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