

REGULATORY MECHANISMS IN CELL-MEDIATED
IMMUNE RESPONSES

III. *I*-Region Control of Suppressor Cell Interaction with
Responder Cells in Mixed Lymphocyte Reactions*

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Alloantigen sensitization in vivo activates in mouse spleen a population of T cells which suppress mixed lymphocyte reactions (MLR) in a noncytotoxic fashion (1). Recently we demonstrated that suppression may be mediated by a soluble factor released into supernates of alloantigen-activated cells which had been restimulated in vitro (2). Antigen specificity of suppressor factor activity for stimulator cell alloantigens was not detected. Rather, suppressor factor did express specificity for the MLR responder cell. Thus, certain strains employed as responder cells were not affected by a soluble factor which markedly inhibited the proliferative responses of other strains, including that syngeneic to the strain producing the factor. Therefore, genetic restriction of suppressor factor interaction with MLR responder cells was suggested.

Cell interactions in T-B-cell collaboration (3, 4) appear to be controlled by the major histocompatibility complex (MHC). The present studies were undertaken to determine whether the suppressor cell interaction with MLR responder cells mediated by a soluble factor is also controlled by the *H-2* complex, and if so by which subregion(s) of *H-2*. The results indicate that the failure of suppression of MLR containing responder cells of certain strains is due to an *H-2*-linked restriction of the suppressor factor interaction with responder cells, and that the suppressor cell-responder cell interaction in the MLR response is controlled by a gene(s) mapped in the *I-C/S* regions of the *H-2* complex.

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 the Texas Inbred Mice Company, Houston, Texas. Mice of the congenic strains B10.A, B10.D2, B10.BR, B10.A(2R), and C57BL/10 were purchased from The Jackson Laboratory, Bar Harbor, Maine.

MLR Culture. MLR culture conditions and techniques utilized for data analysis have been previously described (1).

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Preparations of Suppressor and Control Supernate. Suppressor supernates were produced as previously described (2). Briefly, normal mice were injected into hind foot pads with 2×10^7 allogeneic spleen cells. 4 days later alloantigen-activated spleen cells (hereafter designated with an asterisk, e.g., BALB*) were cocultured 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 cocultures of normal spleen cells with equal numbers of mitomycin C-treated syngeneic cells.

Results and Discussion

Previous studies of the suppression of MLR responses by supernate factors of alloantigen-activated spleen cells suggested that the interaction between suppressor molecules and responding cells was genetically restricted, with *H-2* linkage suggested (2). It was therefore important to rigorously examine genetic constraints on this interaction. Thus, supernate factors were prepared from C57BL/6-activated BALB/c (BALB*) or C57BL/10-activated B10.D2 (B10.D2*) spleen cells and tested in MLR utilizing responder cells which demonstrated different aspects of *H-2* and non-*H-2* genetic similarity with the cells elaborating the suppressor factor. In the presence of BALB/c-derived factor, the responses of both BALB/c and B10.D2 cells were substantially reduced, while addition of the same factor to MLR with C57BL/6 responder cells did not affect the response (Fig. 1, exp. A., groups 1-3). BALB/c and B10.D2 have the same *H-2* complex on different backgrounds, whereas BALB/c and C57BL/6 are dissimilar in both *H-2* and background genetic material. Furthermore, suppressor factor from B10.D2 cells inhibited responses of B10.D2 responder cells but failed to inhibit the response of strain B10.BR cells (Fig. 1, exp. A., groups 4 and 5). B10.D2 and B10.BR are congenic, possessing different *H-2* complexes on an identical genetic background. The results therefore indicate that the genetic restriction of the suppressor factor-responder cell interaction is *H-2* linked, and that mismatch of non-*H-2* background is irrelevant to effective suppression.

Further studies were designed using strains of mice congenic to strain C57BL/10 to determine more precisely the genetic region of the *H-2* complex primarily involved in this model of MLR suppression. Factor produced by C57BL/10-activated B10.D2 spleen cells was tested for suppressor activity in MLR with responder cells of strains B10.D2, B10.A, B10.A(2R), B10.BR, and C57BL/10 (Fig. 1, exp. B). B10.D2 factor suppressed proliferative responses of syngeneic B10.D2 cells (groups 1 and 2), and of B10.A (groups 3 and 4) which shares the *I-C*, *S*, and *D* regions with B10.D2. Strain B10.A(2R) is identical with the *H-2^d* haplotype of B10.D2 only at the *I-C* and *S* regions. Responses of B10.A(2R) (groups 5 and 6) were inhibited as effectively as were those of cells of strains sharing the entire haplotype with the activated suppressor cell. Those strains which share no haplotype regions, B10.BR and C57BL/10, were not suppressed by the B10.D2 factor (groups 7-10). Control supernates did not affect MLR responses in any of the strain combinations tested ($103 \pm 9\%$ control responses). Therefore, genetic homology for as little as the *I-C* and *S* regions was sufficient for active suppression of MLR proliferative responses.

We then investigated whether identities only in the *K*, *I-A*, and *I-B* regions also permitted suppression to be expressed, or whether this was a property unique to the *I-C/S* regions of the *H-2* complex. Suppressor factor was obtained

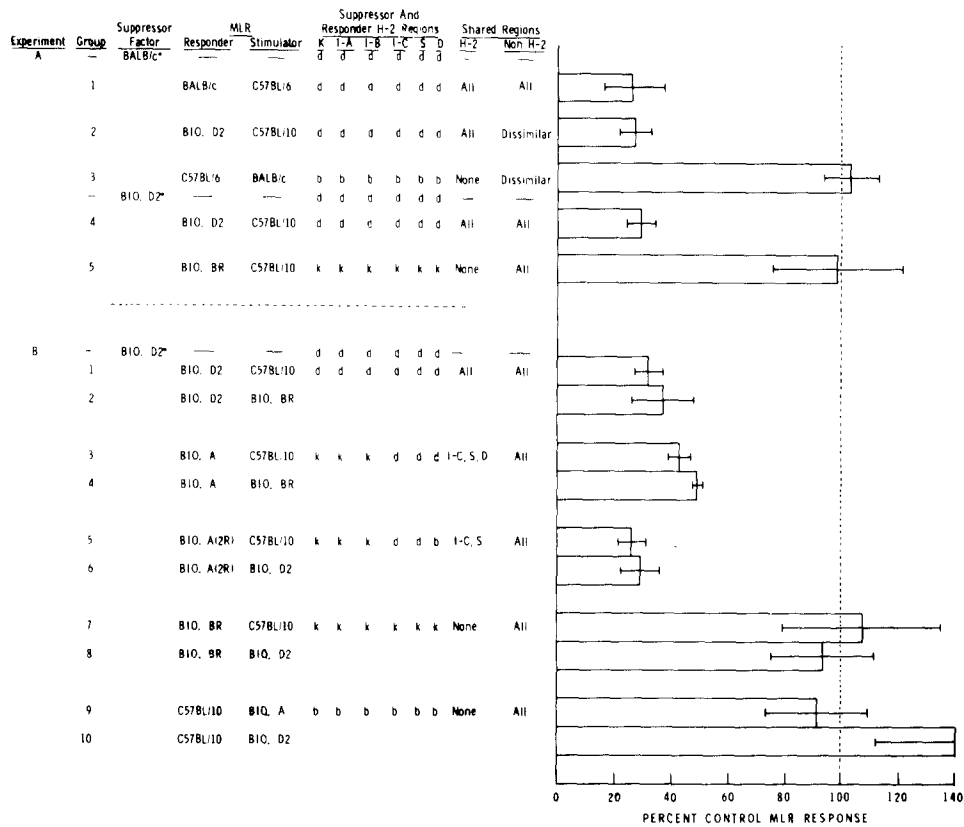


FIG. 1. Activity of suppressor factor from alloantigen-activated suppressor cells in MLR with various responder cell strains. Suppressor supernates from C57BL/6-activated BALB/c (designated BALB/c*) or C57BL/10-activated B10.D2 (B10.D2*) spleen cells were added to MLR of responder and stimulator cell combinations from various inbred and congenic mouse strains (final dilution in MLR, 1:5). Data represent mean responses \pm SEM of four to seven experiments.

from C57BL/10-activated B10.A (B10.A*) spleen cells and tested in MLR with responder cells from B10.D2, which share the *I-C*, *S*, and *D* regions with B10.A, and with responder cells of B10.BR, which differ for these regions but share *K*, *I-A*, and *I-B* with the *H-2^a* haplotype (Fig. 2). B10.A suppressor factor inhibited the response of B10.D2 cells as effectively as the response of syngeneic B10.A responder cells (groups 1, 2, and 5 and 6). B10.A(2R) responder cells which share all regions but *D* were similarly suppressed (groups 3 and 4). In contrast, the response of B10.BR cells, which are identical at the *K*, *I-A*, and *I-B* regions, was unaffected by the same factor (groups 7 and 8). It is important to add that failure to suppress cells of the *H-2^k* haplotype by B10.D2 or B10.A factor did not reflect inability to respond to suppressive signals, since the response of B10.BR (*H-2^k*) cells was suppressed by factor produced by activated *H-2^k* spleen cells (Fig. 2, exp. B.). Moreover, by demonstrating the efficacy of a supernate derived from *H-2^k* cells, this experiment shows that the phenomenon is not peculiar to the *H-2^a* haplotype but probably represents a general feature of regulation of T-T-cell interactions.

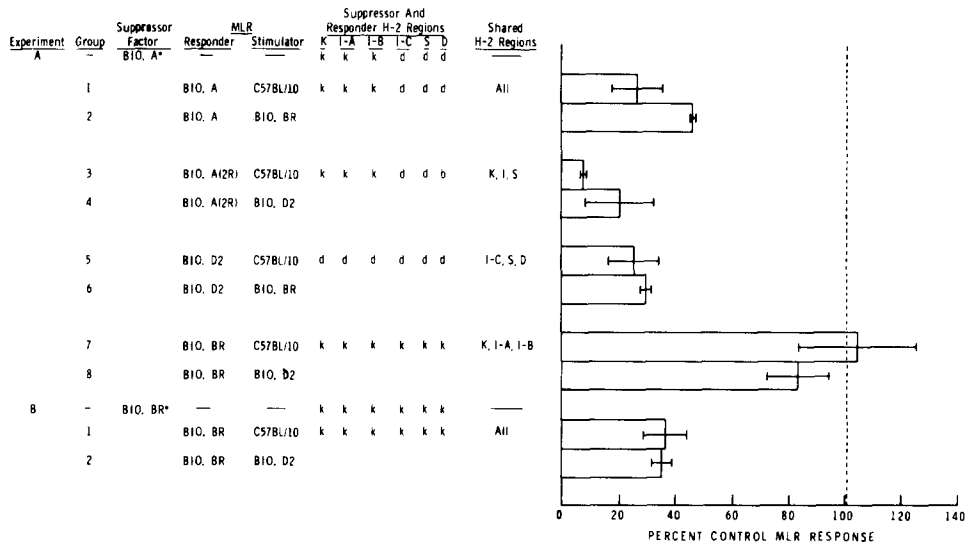


FIG. 2. Activity of suppressor factors from activated suppressor cells in MLR with various congenic responder cell strains. Supernates from C57BL/10-activated B10.A (B10.A*) or C57BL/10-activated B10.BR (B10.BR*) spleen cells were added to MLR of responder and stimulator cell combinations from various congenic strains (final dilution in MLR, 1:5). Data represent mean responses \pm SEM of three to five experiments.

The demonstration with B10.A(2R) cells, that identity for only *I-C* and *S* permits suppression, coupled with identification of genes critical to collaborative cell interactions in antibody synthesis in other subregions of the *I* region, implicate the *I-C* subregion in control of this *T-T*-cell regulatory activity. Presently the *S* region appears not to be involved in control of cell membrane alloantigens, specific immune responses, or *T-B*-cell collaboration, although there is emerging identification of *S*-region control of complement components in the immune response (5). The *I-C* region contains a gene(s) which codes for *I*-region-associated (*Ia*) alloantigen expression (6). In addition, several observations suggest a lymphocyte-activating determinant (*Lad*) locus in the *I-C* subregion (7-9). Recently, one of two *Ir* genes controlling responsiveness to *L-Glu, L-Lys, L-Phe* has been mapped to the chromosomal segment between *I-B* and *D* (10). Genes controlling efficient *T-B*-cell collaboration (11) have not been identified in this subregion. The intriguing possibility may be raised that the *I-C* subregion expresses a function unique to interactions of *T* cells with other functionally distinct subsets of *T* cells.

Mapping of genetic control of suppressor-responder cell interaction in MLR to the *H-2* complex permits speculation about the nature of the active suppressor molecule. Since genetic identity for certain regions of the *H-2* complex is apparently required for suppression, it would seem that similarly identical products of genes located in those regions are involved. Consequently, an homology interaction between the suppressor molecule and a similar molecular configuration expressed by the responding cell may be postulated. As described earlier, the *I-C* region determines expression of certain *Ia* antigens. Whether the supernate of alloantigen-activated cells contains *Ia* moieties is not presently

known. However, complex molecules containing such *I*-region products have been identified in other T-cell factors which mediate regulatory cell interactions in antibody synthesis (12-14). Although MLR responder cells have been reported to lack Ia antigen (7), it is possible that such molecules are exposed or altered to a definable configuration subsequent to antigen stimulation, providing the appropriate site of interaction with an Ia-containing suppressor factor. Preliminary data from this laboratory support such an hypothesis.

These studies demonstrate *I*-region control of T-cell interactions which lead to suppression of immune reactivity. The relationship of the central role of the *I* region in controlling regulatory cell interactions of Ia alloantigens and *Ir* genes is particularly interesting. Further analysis of the nature and functional mechanisms of the suppressor factor will aid continuing redefinition of concepts of *I*-region functions.

Summary

Active suppression of mixed lymphocyte reaction (MLR) response is mediated by a soluble factor released by alloantigen-activated murine suppressor cells. Genetic restrictions controlling suppressor factor interaction with MLR responder cells were elucidated in this study. Non-*H-2* genetic background was irrelevant to effective interaction. Using congenic strains and strains with intra-*H-2* recombinants the genetic locus controlling suppressor T-cell-responder cell interaction was mapped in the *I-C* or *S* regions of the *H-2* complex. Similarly, recombinant strains were used to exclude the presence of another suppressor cell-responder cell interaction locus in *K*, *I-A*, and *I-B* regions. It thus appears that the *I-C* subregion of the *H-2* complex controls suppressive cell interactions in this T-cell-mediated immune response.

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