REGULATORY MECHANISMS IN CELL-MEDIATED IMMUNE RESPONSES

VI. Interaction of *H-2* and Non-*H-2* Genes in Elaboration of Mixed Leukocyte Reaction Suppressor Factor*

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Gene products of the major histocompatibility complex (MHC)[1] participate in T-cell responses in a fashion which provides fundamental recognitive information to interacting cells, either among the T-cell subpopulations, or between macrophages or B cells and T cells (1). Stimulation of distinct T-cell regulatory pathways, i.e., T-cell-mediated help or suppression, may involve selection of the relevant regulatory cells via recognition and/or display of specific MHC products. Thus helper T-cell activity in secondary immune responses to a variety of antigens has been uniquely associated with the *I-A* subregion of the murine MHC (2–5), whereas suppressor function has been associated with the *I-J* (6, 7), or *I-C* (8, 9) subregions.

Consonant with these observations, we have previously reported H-2 restriction among interacting cells in suppression of proliferative responses in mixed leukocyte reactions (MLR) by alloantigen-stimulated T-suppressor cells (8, 10). Syngenicity between responder and primed suppressor cells for the *I-C* subregion of the *H-2* complex permits optimal suppression, as mediated by a soluble factor derived from allosensitized suppressor cells. Furthermore, preliminary data suggest that the suppressive activity of the T-cell factor is functionally dependent upon a product of the *H-2* complex, since exposure to immunoadsorbants prepared with antisera with specificity for the *I-C* and *S* regions of the suppressor cell haplotype removes suppressor activity (9). Previous studies indicated that non-*H-2* gene background had no apparent role in this *H-2*-directed regulatory cell interaction (8). However, in this report identification of a strain unique in its lack of activity in the MLR suppressor system suggests that this role is not exclusively governed by the MHC but rather reflects more complex genetic control. Thus it is demonstrated that non-*H-2*-linked genes exert a dominant permissive function required for MLR suppression by alloac-

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; FCS, fetal calf serum; MEM, Eagle's minimal essential medium; MHC, major histocompatibility complex; MLR, mixed leukocyte reaction.

tivated T cells. Expression of the non-*H-2* gene defect occurs at the level of production of MLR suppressor factor, while expresion of receptors for suppressor molecules is unaltered in the affected strains. Thus, it appears that *H-2* and one or more non-*H-2* genes interact in the final expression of this regulatory T-cell function.

Materials and Methods

Mice. BALB/c mice were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, Tex. Inbred strains C57BL/6J, C57BL/10SN, A.BY, and (BALB/c × C57BL/6J)F_1 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. The congenic resistant strain B6.C/a-H-2^d/By was also obtained from The Jackson Laboratory through the generosity of Dr. Donald Bailey. The F_1 hybrid strain (BALB/c × CBA)F_1 was bred in our animal facility.

MLR. MLR were prepared as previously described (10). 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) with 10% fetal calf serum (FCS) (Reheis Chemical Co., Chicago, 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.

Data from separate 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). E-C from grouped replicate experiments represent mean E-C from three experiments. Percent control MLR response was calculated according to the following formula:

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

Data were analyzed statistically by the two-tailed Wilcoxon rank sum test.

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

Preparation of Absorbing Cells. Concanavalin A (Con A)-stimulated cells to be used as cellular adsorbants were prepared by incubating thymocytes with Con A (3 μg/ml) at 10^7 cells per ml in supplemented MEM containing 5% FCS under Mishell-Dutton conditions (11) for 48 h. At the time of adsorption, cultured cells were harvested and washed three times with 0.15 M methyl-α-D-mannoside and once in Hanks' balanced salt solution.

Absorption of Supernates. Suppressor and control supernates were incubated with 2.5–3.0 × 10^8 packed adsorbing cells per milliliter fluid at 4°C for 30–40 min with frequent mixing. Thereafter the cells were removed by centrifugation.

Results

Failure to Produce MLR Suppressor Factor in C57BL/6J Strain. In the course of previous studies of production and activity of MLR suppressor factor by alloantigen-activated murine spleen cells, a number of inbred and congeneic strains were studied and all produced active factors when tested with syngeneic responder cells in MLR. These strains, including BALB/c, DBA/2, A, C3H,

CBA, AKR, C3H.OH, B10.D2, B10.BR, B10.A, and B10.A(5R) encompass a variety of *H-2* haplotypes and non-*H-2* background genetic composition. However, an exception to the apparently general capacity to produce suppressor factor in response to allogeneic stimulus was revealed in the strain C57BL/6 (B6) (Fig. 1). Factors prepared from B6 in the usual fashion failed to suppress the response of syngeneic B6 responder cells in 21 of 22 supernatant preparations. Variation of allogeneic stimulus used for factor generation, strain of responder or stimulator cells used to assay factor activity, and immunization schedule failed to reveal any suppressive activity (data not shown).

To determine if failure of suppressor activity was associated with the $H\text{-}2^b$ haplotype possessed by B6, we tested the suppressive capability of other strains bearing $H\text{-}2^b$ (Table I). As previously demonstrated, factor produced by B6 did not suppress MLR responses of syngeneic B6 or *H-2* identical C57BL/10 (B10) cells. In contrast, supernates of activated B10 cells suppressed responses of B10 as well as $H\text{-}2^b$ identical B6 and A.BY cells. The same factor had no effect on *H-2* dissimilar BALB/c responder cells.Similarly, another $H\text{-}2^b$ strain of a different background, strain A.BY, suppressed responses of syngeneic as well as *H-2*-matched MLR responder cells while not inhibiting responses of *H-2* nonidentical BALB/c cells. BALB/c responder cells are, however, suppressed by syngeneic factor. Thus, the suppressor defect seems not to be associated with the $H\text{-}2^b$ haplotype in general, but may instead be linked with B6 non *H-2* background genes. Furthermore, since B6 responder cells were capable of suppression by active *H-2*-matched factors, the receptor for suppressive signals on the responder cells appears to be intact. Therefore, the B6 suppressor defect affects specifically the production or subsequent activity of suppressor factors.

Association of Suppressor Defect with B6 Non-H-2 Background Genes. To determine whether inability of B6 cells to product MLR suppressor factor is due to the influence of genes present in the non-*H-2* gene complement, we tested the suppressive capacity of a strain congeneic to C57BL/6, B6.C-$H\text{-}2^d$ (B6.C). This strain possesses an $H\text{-}2^d$ haplotype, rather than $H\text{-}2^b$, on the B6 background. Factors derived from alloactivated B6.C-$H\text{-}2^d$ suppressed neither syngeneic B6.C nor *H-2* identical BALB/c responses (Table II). Furthermore they did not suppress *H-2* dissimilar but background matched B6 responses.In contrast, factor derived from BALB/c ($H\text{-}2^d$) suppressed MLR responses of B6.C as effectively as those of syngeneic BALB/c. Factors derived from background identical B6, or from B10, which possesses a highly similar background and is suppressive in syngeneic responses, had no effect on B6.C responses. Thus genes present in the B6 background appear to prevent or fail tȯ support production of active alloantigen-stimulated suppressor factor.

Genetic Character of Non-H-2 Gene Action in MLR Suppression. To assess the genetic character of the B6 suppressor defect, we investigated the expression of suppressor activity by an F_1 hybrid of a suppressor strain with the nonsuppressor B6 strain. Initially, however, it was necessary to establish the genetic expression of suppressor activity in F_1 hybrids of histoincompatible strains which each showed suppressor activity (Fig. 2). Thus suppressor factor was prepared from (BALB/c × CBA)F_1 ($H\text{-}2^{k/d}$) and tested in MLR with syngeneic F_1, semisyngeneic parental BALB/c or CBA, or unrelated B6 responder cells. F_1 factor suppressed F_1 responses as well as responses of both parental strains,

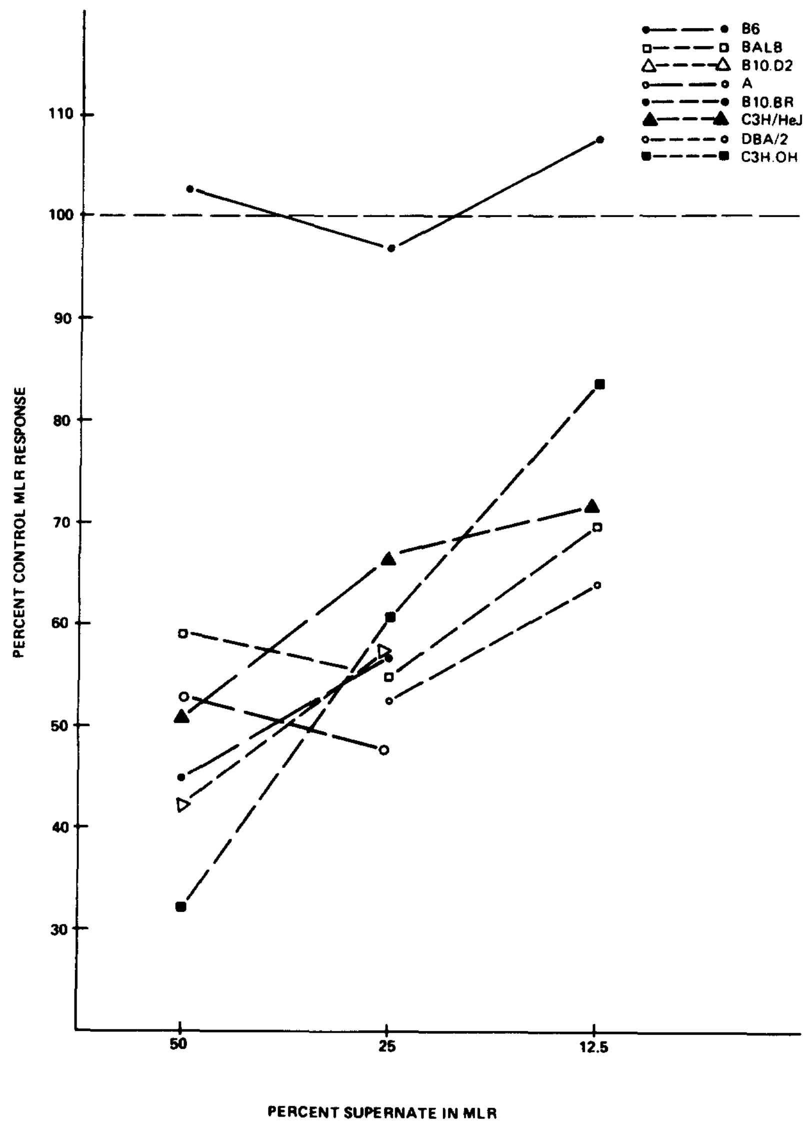

FIG. 1. Activity of MLR suppressor factors derived from alloantigen-activated spleen cells of various strains and tested in MLR with syngeneic responder cells. Data represent mean responses of 3–22 experiments.

while having no suppressive effect on B6 responses (Group 1). Since factor derived from either parental strain was not suppressive of responses of the opposite parent (Groups 4 and 5) it was concluded that suppressor molecules reactive to both parental strains are codominantly expressed in F_1 suppressor T cells. To determine whether such a suppressive activity for parental responses is associated with a single hybrid molecule or rather with separate haplotype specific molecules, adsorption studies were performed (Groups 2 and 3). We have previously demonstrated that Con A-activated thymocytes can specifically

TABLE I
MLR Suppressive Activity of H-2^b Factors

Suppressor strain	Responder strain	cpm (E-C)*		Control MLR response
		Control	Suppressor	
				%
B6	B6	3,872 ± 445	4,616 ± 153	119
	B10	2,878 ± 58	2,590 ± 237	90
B10	B10	2,758 ± 131	1,444 ± 159‡	52
	B6	5,025 ± 611	2,400 ± 486‡	52
	A.BY	5,289 ± 370	3,649 ± 187§	69
	BALB	3,584 ± 260	3,846 ± 862	107
A.BY	A.BY	6,734 ± 359	2,600 ± 138‡	39
	B6	1,323 ± 146	474 ± 71‡	36
	B10	1,301 ± 9	658 ± 79‡	51
	BALB	3,905 ± 454	3,946 ± 419	90
BALB	BALB	4,272 ± 268	2,742 ± 129‡	54

* Net cpm of MLR cultures containing supernates of unstimulated (control) or alloactivated (suppressor) spleen cells at 20% final concentration. Data derived from two to five experiments.
‡ Significantly different from cultures containing control supernate. ($P < 0.01$) by the two-tailed Wilcoxon rank sum test.
§ Significantly different from relevant control ($P < 0.05$).

TABLE II
Failure of Suppressor Factor Activity of B6.C-H-2^d Spleen Cells

Suppressor strain	Responder strain	Shared regions		CPM (E-C)*		Control MLR response
		H-2	Non-*H-2*	Control	Suppressor	
B6.C (*H-2^d*)	B6.C (*H-2^d*)	All	All	1,915 ± 251	2,443 ± 439	128
	BALB (*H-2^d*)	All	Dissimilar	3,745 ± 469	4,145 ± 349	111
	B6 (*H-2^b*)	Dissimilar	All	5,377 ± 529	5,264 ± 544	98
BALB (*H-2^d*)	BALB (*H-2^d*)	All	All	4,540 ± 389	2,613 ± 232‡	58
	B6.C (*H-2^d*)	All	Dissimilar	2,124 ± 131	1,185 ± 81‡	56
	B6 (*H-2^b*)	Dissimilar	Dissimilar	4,978 ± 571	5,584 ± 271	112
B6 (*H-2^b*)	B6 (*H-2^b*)	All	All	5,410 ± 522	5,674 ± 639	105
	B6.C (*H-2^d*)	Dissimilar	All	2,464 ± 470	2,793 ± 387	113
B10 (*H-2^b*)	B10 (*H-2^b*)	All	All	3,638 ± 244	2,019 ± 210‡	55
	B6 (*H-2^b*)	All	Similar	5,024 ± 405	2,400 ± 486‡	48
	B6.C (*H-2^d*)	Dissimilar	Similar	1,640 ± 410	1,527 ± 138	93

* Net cpm of MLR cultures containing supernates of unstimulated (control) or alloactivated (suppressor) spleen cells at 12.5% final concentration. Data derived from two to five experiments.
‡ Significantly different from relevant control ($P < 0.01$).

remove suppressor activity from a suppressor cell supernate (10). Adsorption is effective only when the adsorbing cells are *H-2* compatible with the factor-producing suppressor cell. Therefore F_1 factor was incubated with Con A-activated parental CBA thymocytes and suppressor activity of the adsorbed factor was determined. Such adsorption removed suppressive activity of the F_1 factor for the CBA (*H-2^k*) response, whereas activity for the BALB/c (*H-2^d*) response was unaffected (Group 2). Conversely, adsorption with an *H-2^d*-

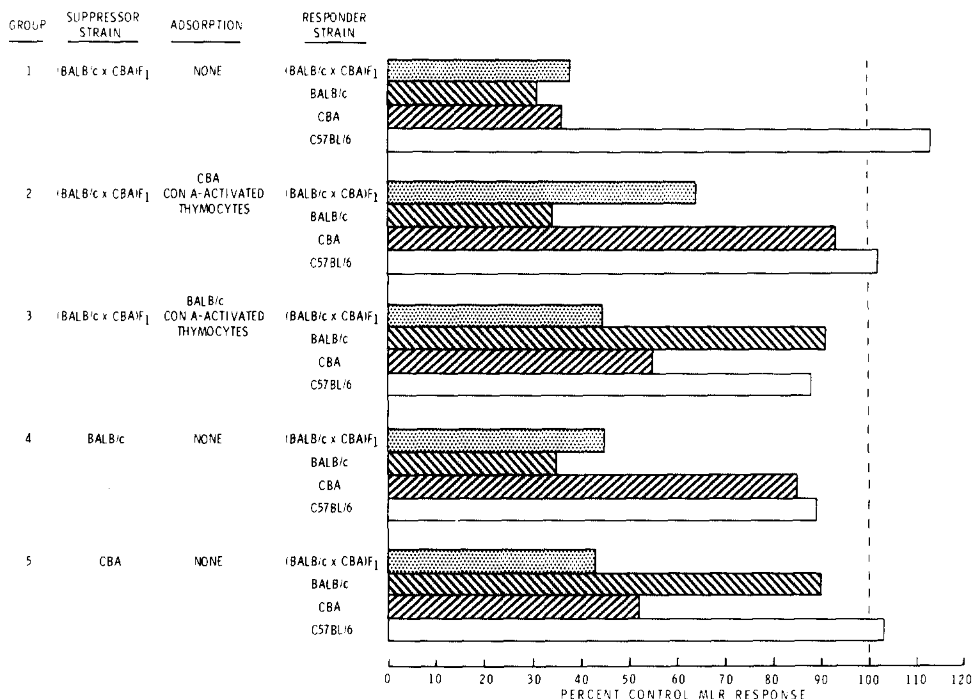

FIG. 2. Activity of MLR suppressor factors from (BALB/c × CBA)F_1 and parental BALB/c and CBA cells in MLR with F_1, parental, or *H-2* unrelated responder cells. Supernates from B6-activated (BALB/c × CBA)F_1, BALB/c or CBA spleen cells were added to MLR without additional treatment or adsorbed with the thymocyte preparations indicated before addition to MLR. Final supernatant concentrations in MLR, 25%. Data represent mean responses of two to six experiments.

specific adsorbant (Con A-activated BALB/c thymocytes) removed suppressive activity for the BALB/c (*H-2*^{d}) response while leaving activity in CBA (*H-2*^{k}) responses intact (Group 3). Each adsorption reduced activity of the F_1 supernate in F_1 responses. The data thus supported codominant expression and production of distinct, parental *H-2* haplotype-specific suppressor molecules by F_1 suppressor cells. In addition, responses of (BALB/c × CBA)F_1 cells were suppressible by either BALB/c or CBA factors, indicating that receptors for both factors are also codominantly expressed on F_1 responder cells in MLR.

With these observations as reference, factors derived from the F_1 hybrid of a suppressor strain, BALB/c, with the non-suppressor strain B6 were tested in an analogous manner (Fig. 3). F_1 factor suppressed responses of syngeneic F_1 as well as responses of BALB/c in MLR (Group 1). Moreover, the same F_1 factor suppressed parental B6 responses entirely as well as BALB/c responses. *H-2*-mismatched CBA responses were not suppressed. F_1 factor was then adsorbed with Con A-activated thymocytes of each parental strain to determine whether F_1 suppressor activity, particularly on the B6 parental responder cell, derived from a parental *H-2*-specific molecule, as suggested by the (BALB/c × CBA)F_1 experiments, or rather via a nonspecific effect. Con A-activated BALB/c thymocytes removed suppressor activity against the BALB/c parent as well

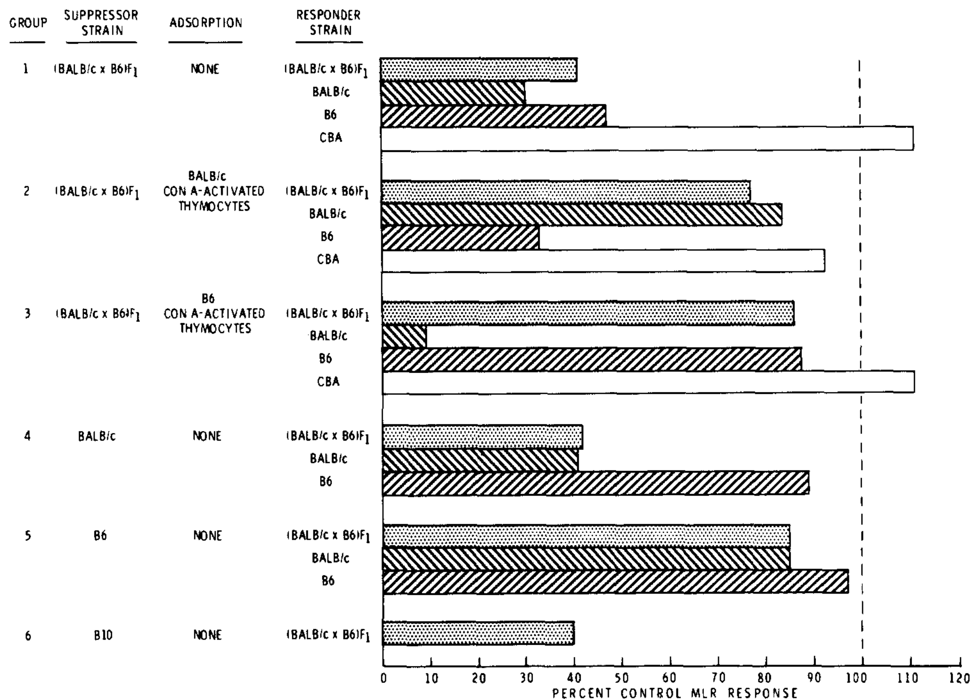

FIG. 3. Activity of MLR suppressor factors from (BALB/c × B6)F_1, parental BALB/c and B6, and B10 cells in MLR with F_1 parental or *H-2* unrelated responder cells. Supernates from CBA-activated (BALB/c × B6)F_1, BALB/c, B6 or B10 spleen cells were added to MLR with no treatment or after adsorption with the thymocyte preparations indicated. Final concentration of supernate in MLR, 25%. Data represent mean responses of two to eight experiments.

as the F_1, leaving suppressor activity against the B6 parent intact (Group 2). Conversely, when a B6 adsorbing population was used, suppressive activity toward B6 was removed while suppression of the BALB/c parental responder remained (Group 3). Therefore, production of the suppressive T-cell factor per se is a genetically dominant trait. It appears that a gene or genes provided through the BALB/c parental non-*H-2* gene complement has permitted the production of a B6-reactive factor. In reciprocal studies with parental or *H-2* homologous factors on (BALB/c × B6)F_1 responder cells, the codominant expression of suppressor receptors coded by B6 as well as BALB/c haplotype was confirmed. Thus BALB/c factor suppressed the F_1 response (Group 4), while, as previously, the B6 factor was ineffective (Group 5). Factor derived from B10, sharing *H-2*^{b} with the B6 partner of the F_1, elicited good suppression (Group 6).

Discussion

Past studies of the MHC in effective suppressor-responder cell interactions in MLR assigned to its products an essential function in promotion of such interactions (8-10). The present results extend comprehension of the genetic interactions underlying such functional restrictions. These results suggest that

gene(s) located in the non-*H-2* gene complement play a consequential role in expression of *H-2*-encoded cell recognition or interaction molecules. Other investigators have described non-*H-2* gene effects in antibody responses to a variety of antigens, some under *Ir* gene control (12–17). The present data represent the initial report of interaction between *H-2* and non-*H-2* genes in regulation of a cell-mediated immune response.

The mouse strain C57BL/6J was identified as exceptional in its inability to produce suppressor molecules in response to allogeneic stimulus, under conditions which elicit suppressor activity in all other strains tested. *H-2* gene malfunction was ruled out and non-*H-2* gene action supported by two observations. While B6 possesses the $H\text{-}2^b$ haplotype and fails to suppress, strains C57BL/10 and A.BY, both also $H\text{-}2^b$, successfully produce factors with suppressive activity. The B6 defect could represent a mutational event of the controlling *H-2* locus which would abrogate the appropriate activity only in the B6 strain. However, a B6 congeneic strain, B6.C-$H\text{-}2^d$, also failed to produce an active factor, thus implicating one or more genes assigned to the non-*H-2* background shared by B6 and B6.C-$H\text{-}2^d$. While neither B6 congeneic strain produced suppressor molecules, both responded to active suppressor factors derived from *H-2* identical strains. Thus the action governed by the non-*H-2* gene(s) does not affect expression of the receptor for suppressor molecules. It may be postulated that the non-*H-2* genes function in a regulatory capacity in the expression or function of suppressor molecules whose structure is coded by *H-2* genes. The same genes do not act in expression of receptor molecules, which may involve products of the same *H-2* gene.

Studies with F_1 hybrid strains were revealing in characterization of inheritance of the regulatory trait as well as of the phenotypic expression of suppressor factors and their receptors. Suppressor cells of F_1 hybrids of *H-2* incompatible strains, each capable of suppression, produced two distinct specific suppressor moieties. Each was functionally complete in the absence of the other as demonstrated by selective adsorption. Therefore *H-2*-coded MLR suppressor molecules are codominantly expressed. Likewise, the display of receptors for suppressor molecules on F_1 responding cells is codominantly expressed, and suppressor factors derived from one parental strain suppressed responses of the F_1 while having no effect on responses of the opposite *H-2* incompatible parent. Since both parental strains BALB/c and CBA, actively suppress appropriately *H-2*-matched MLR responses, it may be assumed that the relevant non-*H-2* genes in these strains are functional. In the F_1 hybrid of BALB/c with the strain B6, in which this gene action does not allow suppressor activity, suppressor molecules specific for each of the parental strains, B6 as well as BALB/c, were again produced. It thus appears that non-*H-2* genes of the BALB/c parent acted in a genetically dominant fashion to fulfill the function required for expression of B6 suppressor molecules. Furthermore the function controlled by this gene appears not to be strain or *H-2* haplotype specific. BALB/c non-*H-2* genes interacted with $H\text{-}2^b$-coded products as effectively as with those of $H\text{-}2^d$.

Identification of the suppressor defect in B6 mice allows a more comprehensive view of genetic control of suppressor cell interactions in MLR. Several

genetic and functional postulates may follow from the phenomena described. Thus multiple genes control the phenotypic expression of suppressor cell activity, with at least one gene coded in the *I-C* subregion of the murine MHC and at least one gene mapped to the non-*H-2* portion of the genome. A regulatory function might be postulated for the non-*H-2* gene, allowing expression or subsequent function of suppressor molecules. Such a gene may be instrumental in the assembly of a complex regulatory molecule composed of multiple subunits; alternatively it may be a structural gene for one of the subunit moieties. Preliminary observations suggest that Ia molecules may contribute to the function of MLR suppressor factor (9). Murine Ia alloantigens are composed of two different polypeptide chains, only one of which is required to bear antigenic determinants (18). Among several hypotheses it has been suggested that the second chain could be encoded in distinct, or unlinked genetic regions, and that it might subserve either an antigen recognition function or modify the biological function of the Ia chain. Thus the non *H-2* gene here described could be acting at the level of Ia molecular composition. Biochemical assessment of molecules precipitated by the appropriate anti-H-2^b antisera might be revealing in this regard. Finally this gene may affect cellular release or secretion of suppressor molecules, although preliminary experiments, in which sonicates rather than supernates of alloactivated B6 cells have been tested, disclosed no suppressive activity.

These data may also suggest that distinct non-*H-2* genes control expression of suppressor factor molecules and the receptors for those molecules. While B6 and B6 congeneic mice fail to produce B6-reactive suppressor factors, they do express receptors for active *H-2*^{b} factors. Failure of receptor expression has not yet been identified in the MLR suppressor system. Taniguchi et al. (19) have made similar observations of non-*H-2* gene effect on *H-2*-coded suppressor molecule function in response to KLH. In addition, they have identified distinct non-*H-2* gene action in expression of receptors for KLH-specific suppressor factors. In the latter instance, congeneic lines of B10 background fail to express the receptor while they produce the factor normally. The closely related B6 strain has no defect. A similar B6-B10 distinction exists at the level of suppressor molecule activity in the MLR suppressor model. Thus, separate but perhaps physically linked non-*H-2* genes which influence *H-2*-encoded suppressor functions in both systems may map to that part of the genome which differentiate B6 and B10.

Of many genetic markers which have been identified for the related B6 and B10 strains, including T-cell expressed markers such as Ly alloantigens and G_{IX}, only two systems differentiate the two strains, *H-9* and a locus controlling levulinate hydratase which are both unmapped (20). While the non-*H-2* gene effect in MLR suppression similarly differentiates these strains, it is possible that the various B6 sublines may also show regulatory differences with regard to MLR suppression. Such B6 intra-line distinctions have been noted in multigenic influences on Ir gene controlled responses to terpolymers of glutamic acid, lysine, and alanine (14).

Finally if one invokes the phenomenon of associative recognition as the basis for *H-2* restriction of MLR suppressor activity, the B6 non-*H-2* gene defect

might be expressed at two levels. If a requirement for *H-2* identity between primed suppressor cells and MLR responder cells at the *I-C* subregion reflects in fact initial sensitization of suppressor T cells to foreign alloantigens in the context of self molecules encoded by *I-C*, it is possible that in the B6 strain either the adaptor/interaction molecule is defective, preventing an immunogenic stimulus, or the T-suppressor cell receptor specific for the syngeneic *I-C* product is defective, preventing recognition. Data do not currently exist in the MLR suppressor system to support or deny the concept of associative recognition, but such data from other cell interaction systems suggest that it is an important consideration (21-26).

Reports from other investigators have described inhibition of MLR and cytotoxic lymphocyte generation by allosensitized B6 cells (27-29). Initial efforts in our laboratory to compare regulatory effects of B6 cells with soluble factors produced by those same cells have revealed a somewhat paradoxical profile of activity. While B6 factors are inactive, inclusion of alloactivated B6 cells into MLR with syngeneic responder cells leads to antigen-specific depression of the response (S. Rich and D. Kastner, unpublished observations). In our previous studies of alloactivated regulatory cells in MLR (30) and cytotoxic lymphocyte generation (31), the various strains tested produced suppression which was antigen *non*specific, in parallel with antigen nonspecificity of suppression mediated by soluble factors. The discordance between activities of B6 factors and intact B6 cells, as well as with the activities of other strains, may reflect an aspect of specific suppression which is masked by antigen nonspecific effects predominant in other strains. Alternatively, antigen-specific cytotoxic activity of alloactivated B6 cells against MLR stimulator cells may play a role, although it has not been demonstrated in previous approaches to this question (31).

Clearly, regulatory T-cell functions involve complex cellular interactions as well as multiple and probably nonexclusive mechanisms for achieving these interactions. Gene products of the MHC provide recognitive information which is essential to many interactions which have been studied. Continuing identification of genetic control mechanisms will provide insights not only into the mechanisms underlying phenotypic expression of MHC gene products but may also provide clues regarding the diversity of expression of different MHC genes on functionally distinct subsets of T cells.

Summary

Previous studies have shown that alloantigen-activated spleen T cells produce a soluble factor which suppresses mixed lymphocyte reaction proliferative responses, and that the interaction between suppressor and responder cells is controlled by genes of the *H-2* complex. However a defect in the expression of suppressor activity was identified in the mouse strain C57BL/6J. Factor prepared from alloactivated B6 spleen cells failed to suppress MLR responses of syngeneic or *H-2* compatible responder cells. Unimpaired suppressor factor production by other $H\text{-}2^b$ strains and failure of suppressor factor production by a B6 congenic strain, B6.C-$H\text{-}2^d$ isolated the defective gene to the non-*H-2* portion of the genome. In addition, the defect appeared to be related specifically to inability to produce an active factor, while the capacity to respond to

suppressor molecules was unimpaired. The genetic character of the non-*H-2* gene action was identified in F_1 hybrid studies. Initially F_1 hybrids of the nondefective histoincompatible strains were studied. Suppressor factor from F_1 cells suppressed the responses of both parental strains, and parental factors each suppressed the response of F_1 cells. Adsorption of F_1 factor with Con A-activated thymocytes of either parental strain removed suppressor activity specific for that strain, leaving activity against the other parental strain intact. The data support codominant expression and production of distinct, parental *H-2* haplotype-specific suppressor molecules by F_1 suppressor cells. An F_1 hybrid of the defective B6 strain with nondefective BALB/c produced suppressor factor which was also capable of suppressing both parental strains. Production of a suppressive B6-reactive factor by F_1 cells was verified by adsorption studies. Thus it appears that non-*H-2* genes of the BALB/c parent acted in a genetically dominant fashion to provide the function required for expression of B6 suppressor molecules. We conclude that multiple genes control the expression of alloactivated suppressor cell activity, with at least one gene mapped to the *I-C* subregion of the murine major histocompatibility complex and one or more genes mapped to the non-*H-2* gene complement.

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