Brief Definitive Report

REGULATORY MECHANISMS IN CELL-MEDIATED IMMUNE RESPONSES

V. *H-2* Homology Requirements for the Production of a Minor Locus-Induced Suppressor Factor*

By DANIEL L. KASTNER, ROBERT R. RICH,‡ LILY CHU, AND SUSAN SOLLIDAY RICH

(From the Departments of Microbiology and Immunology and Medicine, Baylor College of Medicine, and the Methodist and Veterans Administration Hospitals, Houston, Texas 77030)

Earlier work from this laboratory has shown that mice subcutaneously immunized with *H-2*-disparate lymphocytes give rise to a population of splenic T cells which suppress the proliferative response in mixed leukocyte reactions (MLR) (1). When these suppressor cells are restimulated in vitro with the antigen used for in vivo immunization, they produce a soluble mediator capable of suppressing the MLR (2). Factors generated in this way have been shown to suppress the MLR proliferation of only those cells which share the *I-C* subregion of the major histocompatibility complex (MHC) with the factor-producing cells (3). However, alloantigen-induced suppressor factors show no apparent antigen specificity: they inhibit the proliferation of *I-C* homologous lymphocytes to any alloantigen (2).

In this report we extend our previous observations by showing that MLR suppressor function can be stimulated by non-H-2 histocompatibility antigens. In studies of the sensitization of cytotoxic T lymphocytes to minor histocompatibility determinants, Bevan (4), and Gordon et al. (5) have established a fundamental role for MHC-encoded antigens in the recognitive process. Therefore, we investigated the possibility that the *H-2* haplotype of the in vitro restimulating cell might similarly determine whether minor locus-induced suppressor cells produce suppressor factor. We found that splenocytes taken from an animal primed with *H-2*-identical, minor locus-disparate cells would produce suppressor factor only if the in vitro restimulating cells shared both H-2 and non-H-2 antigens with the primary stimulators.

Materials and Methods

Mice. BALB/c ($H\text{-}2^d$) mice were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, Tex. DBA/2J ($H\text{-}2^d$), DBA/1J ($H\text{-}2^q$), D1.C/Sn ($H\text{-}2^d$, a congenic resistant strain of DBA/1 background with BALB/c *H-2* complex), and CBA/J ($H\text{-}2^k$) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Culture Medium. Cells were cultured in Eagle's minimal essential medium with Hanks' salts (Microbiological Associates, Bethesda, Md.) supplemented with 10% fetal calf serum (lot A561222,

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‡ Investigator, Howard Hughes Medical Institute.

Grand Island Biological Co., Grand Island, N.Y.) and gentamicin, sodium pyruvate, sodium bicarbonate, L-glutamine, and nonessential amino acids as previously described (6).

MLR. MLR cultures and data analysis were performed exactly as detailed in an earlier paper (7).

Preparation of Minor Locus-Induced Suppressor and Control Supernates. Mice were immunized with 1×10^7 *H-2*-identical, minor locus-disparate splenocytes in each rear footpad. From 4 to 14 days later these animals were sacrificed, and their splenocytes were incubated with various mitomycin C-treated stimulator cells in culture medium modified to contain 2% fetal calf serum, as described previously (2). Supernates were harvested (2) and added to MLR at final concentrations of 33, 17, and 9%. Control supernates were derived from co-cultures of normal splenocytes with equal numbers of mitomycin C-treated syngeneic splenocytes.

Results

BALB/c mice were immunized subcutaneously with DBA/2 splenocytes and restimulated in vitro in an attempt to generate a non-H-2-induced suppressor factor (Fig. 1). These two strains differ at numerous minor histocompatibility loci, including the *M* locus (*Mls*), *H-1*, *H-7*, *H-8*, and *H-13* (8). Supernates were assayed on a BALB/c anti-DBA/2 proliferative response, which is generated largely because of the *Mls* difference between the two strains (9). In contrast with H-2-induced MLR suppressor cells (2, 3), DBA/2-sensitized BALB/c spleen cells did not produce appreciably suppressive supernates 4 days after immunization. However, at 7 days postimmunization, supernates produced by DBA/2-sensitized BALB/c cells were markedly suppressive; the net thymidine incorporation in the presence of the 7-day factor was 29% of that obtained in the presence of control factor. As in the experiment shown in Fig. 1, suppressive activity usually diminished by day 10, but was sometimes detectable as late as day 14.

In order to approach the question of whether minor locus-activated suppressor cells recognize MHC-encoded structures, the following experiment was performed. BALB/c mice were sensitized with minor locus-disparate D1.C splenocytes 7 days before sacrifice. Splenocytes from immunized mice were restimulated in vitro with either D1.C or DBA/1 cells, and supernates were obtained. Since DBA/1 and D1.C differ genetically only at *H-2*, restimulation with DBA/1 presents the suppressor cell with all of the minor locus antigens used for in vivo priming, but the restimulating cell is $H\text{-}2^q$ instead of $H\text{-}2^d$. Thus, if factor production requires *H-2* homology between the suppressor cell and the in vitro restimulating cell, or between the in vivo sensitizing cell and the in vitro restimulating cell, then restimulation with D1.C should result in factor production, whereas restimulation with DBA/1 should not.

Inspection of Table I reveals that restimulation with DBA/1 was quite ineffective in generating suppressor factor. Supernates produced in this manner were not suppressive of BALB/c proliferative responses to C57BL/6 at any of the concentrations tested, and were only marginally suppressive of the BALB/c response to CBA at the 33% concentration of supernate. In contrast, restimulation with D1.C led to the production of a supernate which clearly inhibited the BALB/c response to both C57BL/6 and CBA. Thus, D1.C-sensitized BALB/c cells produce suppressor factor only when rechallenged with the immunizing minor locus antigens in conjunction with the appropriate MHC-encoded structures. Once stimulated, though, minor locus-induced factors suppress proliferative

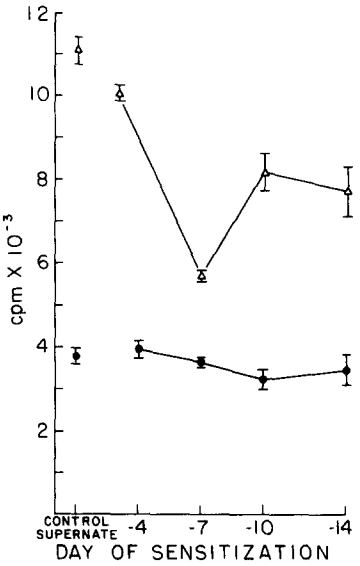

FIG. 1. BALB/c mice were injected in the hind footpads with DBA/2 spleen cells at various intervals before sacrifice. All animals were killed on the same day, and their splenocytes were cultured with mitomycin-C-treated DBA/2 stimulator cells for 24 h. Supernates were then added to BALB/c vs. DBA/2 mixed lymphocyte reactions at a final concentration of 33%. Control supernates were produced as described in Materials and Methods. The abscissa shows the time (in days) between in vivo sensitization and sacrifice. The ordinate gives the thymidine incorporation as counts per minute $\times 10^{-3}$. Data are means ± SEM of quadruplicate cultures. (△) BALB/c stimulated by mitomycin C-treated DBA/2; (●) BALB/c stimulated by mitomycin C-treated BALB/c.

TABLE I

H-2 Homology Requirement for Generation of Minor Locus-Induced Suppressor Factor

Final concentration of factor	A. BALB/c vs. C57BL/6 MLR*			B. BALB/c vs. CBA/J MLR*		
	BALB/c‡	DBA/1§	D1.C‖	BALB/c‡	DBA/1§	D1.C‖
%						
33	4,477 ± 1,262	4,323 ± 625	1,419 ± 254	16,079 ± 206	11,387 ± 327	5,850 ± 304
	(100)	(97)	(32)	(100)	(71)	(36)
17	5,990 ± 1,007	7,804 ± 499	3,576 ± 321	15,945 ± 788	16,631 ± 789	9,063 ± 622
	(100)	(130)	(60)	(100)	(104)	(57)
9	6,682 ± 474	7,890 ± 461	4,887 ± 575	17,935 ± 673	17,236 ± 295	12,068 ± 352
	(100)	(118)	(73)	(100)	(96)	(67)

* Data are expressed as the mean difference between quadruplicate stimulated and unstimulated cultures ± SEM. Numbers in parentheses give the data as a percentage of the proliferation in the presence of BALB/c stimulated supernate.
‡ Supernates obtained by culturing 1×10^7 normal BALB/c cells with an equal number of mitomycin-treated BALB/c cells.
§ Supernates obtained by culturing D1.C-sensitized BALB/c cells with an equal number of mitomycin-treated DBA/1 cells.
‖ Supernatants obtained by culturing D1.C-sensitized BALB/c cells with an equal number of mitomycin-treated D1.C cells.

responses both to the non-*H-2*-linked Mls antigen, as shown in Fig. 1, and to various H-2 specificities, as shown in Table I.

Discussion

Recent studies from our laboratory (1–3, 6, 7) have been concerned with suppressor T-cell activation by H-2-disparate alloantigens. As an extension of

this work, we now describe the conditions for activation of suppressor cells by minor histocompatibility antigens. Our data show that although minor locus-induced suppressor cells do not appear in the spleens of immunized animals as rapidly as do H-2-induced suppressors, there are obvious parallels between the two systems. Both H-2- and minor locus-induced suppressor cells produce MLR suppressor factors when restimulated in vitro with cells from the original sensitizing strain, and in both cases these factors are antigen-nonspecific. Biochemical and immunogenetic similarities or differences between these factors have not yet been established.

Bevan (4) has previously shown that cytotoxic T lymphocytes can be generated against minor histocompatibility antigens, and that such effector cells lyse only those targets bearing both the appropriate minor locus antigens and H-2 antigens homologous with the original stimulator strain. The work presented in this paper shows similar results for suppressor cells: MLR suppressor cells can be stimulated by non-H-2 antigens, but in vitro suppressor factor production is only triggered by cells bearing the proper minor locus determinants and the appropriate H-2 antigens. These experiments do not differentiate between a requirement for *H-2* homology between the in vivo sensitizing cell and the in vitro restimulator and requirement for *H-2* homology between the in vitro restimulator and the factor-producing cell. Studies of one-parent radiation chimeras (10–12) have established that for trinitrophenol- and virus-specific cytotoxic cells, *H-2* gene-homology is required between sensitizing and target cells, but not between effector and target cells.

Other authors have noted the inhibitory effects of injecting mice with *H-2*-identical, *Mls*-disparate cells. Jacobsson et al. (13) found that lymphoid cells from CBA/H ($H\text{-}2^k, Mls^b$) mice sensitized 1–4 wk earlier with C3H ($H\text{-}2^k, Mls^c$) were specifically unresponsive to C3H cells in a proliferative assay. However, in cell-mixing experiments these authors failed to detect any suppressor cell activity. In addition, Matossian-Rogers and Festenstein (14) have shown that lymph node cells from CBA/H mice injected intraperitoneally with BRVR ($H\text{-}2^k, Mls^a$) cells become transiently hyporesponsive when assayed for the in vitro generation of cytotoxic cells against (CBA/H × DBA/2)F_1 ($H\text{-}2^{k/d}, Mls^{b/a}$) cells. It was subsequently shown (15) that this phenomenon is the result of active suppression, and that re-exposure to the immunizing Mls antigen is required for suppressor cell activity. However, the question of *H-2* homology requirements was not addressed.

The experiments described in this communication all involve sensitization across *Mls*: BALB/c possesses the Mls^b allele, while DBA/2, DBA/1, and D1.C all share the Mls^a allele. However, the latter strains differ from BALB/c at several other non-*H-2* histocompatibility loci (8), and as yet we have no direct evidence that the Mls antigen is unique among minor locus antigens in inducing suppressor activity. Whatever the specific antigenic stimulus, though, our data may be taken as part of a growing body of evidence linking the recognition of minor histocompatibility antigens with MHC-encoded structures. Studies of the associative recognition process in minor locus-induced suppressor cells, cytotoxic cells (4), and proliferating cells (16) should yield further understanding of the interrelationships among these lymphocyte subpopulations.

Summary

A mixed leukocyte reaction suppressor factor is produced by spleen cells sensitized in vivo and restimulated in vitro across non-H-2 antigenic barriers. Cells capable of producing this factor appear in the spleens of minor locus-immunized animals later than in animals sensitized to major histocompatibility complex-encoded antigens. However, both H-2 and non-H-2-induced factors suppress proliferative responses to any alloantigen. Splenocytes from animals immunized with *H-2*-identical, minor locus-disparate cells produce suppressor factor in vitro only when restimulated with cells sharing both H-2 and non-H-2 antigens with the in vivo stimulators.

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