Igh VARIABLE REGION-RESTRICTED T CELL INTERACTIONS

Genetic Restriction of an Antigen-specific Suppressor Inducer Factor Is Imparted by an I-J$^+$ Antigen-nonspecific Molecule*

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Regulation of immune responses involves complex interaction between a number of T cell sets (1, 2). These interactions often require a genetic homology between the T cells involved for the functional activity to be manifest. Two gene polymorphisms control genetic restrictions between cells of suppressor T cell circuits. The first major restricting element of suppressor T cell interactions concerns antigens of the major histocompatibility gene complex (MHC).[1] The other restricting element involves genes encoded in or linked to the variable region (V) of the Igh gene loci. The question of how the Igh-V-related gene products involved in T suppressor cell interactions relate to the antigen specificity of the cellular interactions is not well known, although a number of investigators have suggested an idiotype-antiidiotype–like interaction similar to the one originally proposed by Jerne (3).

Investigations into the nature of genetically restricted T cell interactions have been greatly facilitated by the use of cell-free products that replace the activity of functional T cell sets. One such product is an antigen-specific, T cell-derived factor (TsF_1) from antigen-induced, first-order Ly 1 suppressor T cells (Ts_1) that suppresses delayed-type hypersensitivity responses to the hapten, azobenzenearsonate (ABA) (4). The Ly-1$^+$ Ts_1 cells produce a molecule (or molecules) that binds antigen and is recognized by antibodies reactive against I-J and a dominant cross-reactive idiotype (6). Similar TsF_1 molecules have been shown to function by inducing a second set of Ts, termed Ts_2, from resting Ly-1,2 T cell populations. The TsF_1 share serologically detectable idiotypic elements with antibody of the same specificity (5, 6), while the Ts_2 induced by TsF_1 are antiidiotypic (7–9). These Ts_2 act by triggering another set of idiotype-bearing T cells, termed

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[1] *Abbreviations used in this paper:* ABA, azobenzenearsonate; CRI, cross-reactive idiotype; DTT, dithiothreitol; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SE, sheep erythrocytes; Ts, T suppressor cells; TsF, T suppressor factor; TsiF, T suppressor-inducer factor; V, variable region.

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Ts_3, which appear to be the terminal cell in the suppressor pathway (10, 11). These results suggest that the nature of Igh-V restriction lies in a complex series of cellular interactions determined by complementary receptor-antireceptor binding.

Recently, another first-order, antigen-specific T cell factor secreted by immune Ly-1 T cells (Ly-1 TsiF) has been described that suppresses the plaque-forming cell (PFC) response of naive spleen cells to sheep erythrocytes (SE) in vitro (12). Ly-1 TsiF, like TsF_1, is a two chain factor, one chain of which is antigen binding and $I\text{-}J^-$, the other $I\text{-}J^+$ and antigen nonspecific (13). This factor must also interact with a Ly-1,2 target cell to induce suppression. This transducer cell then activates a third cell, a Ly-2 T suppressor effector cell, which results in suppression of Ly-1 T helper cells. Although the nature of idiotypic determinants on these factors is not known, the Ly-1 TsiF also exhibits an Igh-V-linked genetic restriction with its target cell (12). It was found in recombination experiments that the Igh-V-restricting element of the Ly-1 TsiF was the $I\text{-}J^+$ antigen-nonspecific molecule, not the antigen-binding one (13).

In the present set of experiments, we attempted to resolve this question concerning genetic restrictions in the Ts pathway by combining elements of two distinct suppressor T cell systems. The functional similarities between the SE-specific Ly-1 TsiF and the ABA-specific TsF_1 suggested to us that these two molecules served essentially the same purpose: to initiate a set of cellular interactions that results in the antigen-specific suppression of Ly-1 T cell activity. The fact that functional domains of each of these TsF could be separated allowed us to construct "hybrid molecules" by taking antigen-binding material from SE-specific Ly-1 TsiF and $I\text{-}J^+$ antigen-nonbinding material from F12, a T cell hybridoma that secretes an ABA-specific TsF_1 (14). Our results show that the antigen-binding $I\text{-}J^-$ chain from Ly-1 TsiF, when mixed with the $I\text{-}J^+$ chain of the F12 TsF_1, can form a fully functional T suppressor factor that can suppress the anti-SE PFC response of naive spleen cells. This hybrid factor shows an Igh-V-linked genetic restriction in its activity; interestingly, it is the $I\text{-}J^+$ antigen-nonbinding molecule from the ABA-specific TsF_1 that can act as the restricting element. The genotype of the cell that makes the antigen-binding factor appears to be irrelevant; when combined with the F12 $I\text{-}J^+$ chain, this suppressive complex induces antigen-specific suppression in all mice expressing the Igh^e gene polymorphism, the Igh haplotype of A/J mice.

Materials and Methods

Mice. 6–10-wk-old C57Bl/6, BALB/c, A/J, $B6AF_1$, $CB6F_1$, C57Bl/10, and B10.A mice were obtained from The Jackson Laboratory, Bar Harbor, ME. BALB.K, BALB.B, and C.AL20 mice were bred and maintained at the animal facilities of the Yale University School of Medicine.

Antisera. Monoclonal anti-Ly sera were generously supplied by F.-W. Shen, Memorial Sloan-Kettering Cancer Center, New York. Anti-$I\text{-}J^b$ serum was prepared by hyperimmunizing B10.A(5R) recipients with a mixture of B10.A(3R) spleen and lymph node cells (antiserum ASM 20). Anti-$I\text{-}J^k$ serum was prepared by hyperimmunizing B10.A(3R) recipients with a mixture of B10.A(5R) spleen and lymph node cells (antiserum ASM 18; we thank Dr. D. B. Murphy, Yale University, for preparing these antisera). Monoclonal anti-$I\text{-}J^k$ antibody (WF.8.C. 12.8) was prepared as previously described (15) and was a generous gift of Dr. Carl Waltenbaugh, Northwestern University, Chicago, IL.

Antigens. SE were obtained from Colorado Serum Co., Denver, CO. ABA was obtained from Eastman Kodak Co., Rochester, NY.

Method of Cell Preparations. Spleen cells were washed in buffered saline solution and suspended in RPMI 1640 supplemented with antibiotics, 10% fetal calf serum, 100 mM glutamine, 25 mM Hepes, and 5×10^{-5} M 2-mercaptoethanol for tissue culture. Suppressive activity of T cell factors was determined by addition of these materials to cultures of unprimed spleen cells. All cells were suspended in culture medium at a concentration of 1×10^7 spleen cells in 1 ml and cultured with 0.05 ml of a 1% (vol/vol) suspension of SE in Falcon 3008 plates (Falcon Labware, Oxnard, CA) in a 5% CO_2, 95% air incubator for 5 d. The number of PFC was determined on day 5 by using the Cunningham modification of the Jerne-Nordin plaque assay, as previously described (16). Results are given as the mean of three individual calculations from each culture condition.

Preparation of TsF. The generation and characterization of the ABA-specific F12 hybridoma has been previously described (14). The preparation of Ly-1 TsiF (10) involves treatment of a suspension of spleen cells from mice hyperimmunized to SE with anti-Ly-2 antibody and rabbit complement, and subsequently cultivating the cells in vitro for 48 h in tissue culture medium at a concentration of 10^7 cells/ml. After 48 h, supernatant fluids were cleared and passed through Millipore filters (Millipore Corp., Bedford, MA).

Separation of TsF on Anti-I-J Immunosorbents. Supernatants from hybridoma cell lines (reduced and run in 0.3 M borate buffer containing 5 mM dithiothreitol (DTT) [17]) or Ly-1 TsiF were passed over the appropriate immunosorbent column made from anti-I-J sera or anti-I-J monoclonal antibody coupled to Sepharose 4B. After extensive washing, the column was eluted with 0.2 M sodium carbonate (Na_2CO_3), pH 11.0, and immediately neutralized in 0.3 M borate buffer, pH 8.3. The filtrate and eluate were then concentrated back to their original volume and dialyzed overnight, first against phosphate-buffered saline (PBS), then against RPMI 1640.

Results

I-J+ Molecule of Ly-1 TsiF from A/J Mice Imparts the Igh Region Restriction on the Biological Activity of the Ly-1 TsiF. Previous results (13) in B6 mice have shown that the I-J+ antigen-nonbinding subfactor of the Ly-1 TsiF imparts the Igh-linked restriction exhibited by the factor. We sought to confirm this observation in A/J mice. The results in Table I, using Igh and MHC congenic mice as a source of either antigen-binding or I-J+ chain show that the I-J+ molecule and not the antigen-binding one had to come from cells that expressed the same Igh complex as the assay cells for suppression to occur. The fact that neither the antigen-binding molecules nor the I-J+ molecules alone could suppress the assay cells indicates that no contaminants were present in these factor preparations. These results confirm earlier findings in the SE system that the I-J+ molecule mediates the Igh genetic restriction exhibited by Ly-1 TsiF and extends them to include Igh-restricted interactions between cells exhibiting the Igh^e haplotype.

I-J+ Molecule of the ABA-specific T Cell Hybridoma F12 Can Restore the Suppressive Activity of Ly-1 TsiF Depleted of I-J+ Material in the SE System. We then asked whether the I-J+ material secreted by the F12 hybridoma could functionally replace the I-J+ material needed from Ly-1 TsiF biological activity (Table II). An SE-specific Ly-1 TsiF from A/J mice was fractionated into two separate activities on an anti-$I\text{-}J^k$ column. While neither the I-J− filtrate (which contains the antigen-binding material) nor the I-J+ eluate had any suppressive activity, recombination of the I-J− and I-J+ subfactors returned suppressive activity to the Ly-1 TsiF. When supernatant from the F12 hybridoma was passed over an anti-$I\text{-}J^k$ column, the eluted material was also able to return to the Ly-1 TsiF I-J−

TABLE I

I-J$^+$ Chain and Not the Antigen-binding Chain of Ly-1 TsiF Imparts the Igh-V-linked Genetic Restriction in the Igh^r Haplotype

Source of SE-specific antigen-binding chain*	Source of I-J$^+$ material	Anti-SE PFC/culture‡	
		BALB/c	C.AL20
—	—	2,800	1,000
A/J (H-2^a, Igh^c)	—	2,400	1,100
B10.A (H-2^a, Igh^b)	—	3,300	1,200
BALB.K (H-2^k, Igh^a)	—	3,100	1,000
—	A/J (H-2^a, Igh^c)	2,200	900
A/J		1,900	**300**
B10.A		2,900	**400**
BALB.K		2,100	**100**
—	BALB/c (H-2^d, Igh^a)	2,700	1,100
A/J		**1,100**	900
B10.A		**500**	1,100
BALB.K		**600**	900
—	A.By (H-2^b, Igh^c)	3,200	1,200
A/J		4,100	**500**
B10.A		2,700	**400**
BALB.K		2,900	**500**

* I-J$^-$ antigen-binding material was filtrate of Ly-1 TsiF passed over an anti-I-J immunosorbent; I-J$^+$ material was obtained by elution of passed material off this column. Both filtrate and eluate were concentrated to original volume, dialyzed first against PBS, then RPMI 1640, and added at a final concentration of 1:10.

‡ Primary in vitro response to SE. Suppressed PFC responses are shown in boldface.

filtrate the ability to suppress the anti-SE PFC response of B6AF$_1$ spleen cells. The suppressor complex of SE antigen-binding and F12 I-J$^+$ material failed to suppress the B6 spleen cell response, indicating that this suppressor factor still exhibits a genetic restriction. The anti-I-J^k column eluates of supernatant from the F3 hybridoma (a T cell hybridoma that secretes an ABA-binding protein but no detectable I-J$^+$ material and hence has no biological activity [Jonathan Bronberg, Blake Whitaker, and Mark Greene, unpublished observations]) or the BW 5147 thymoma (the cell line used for fusion to generate the F3 and F12 hybridomas) failed to return suppressive activity to the Ly-1 TsiF antigen-binding material. The F12 I-J$^-$ filtrate, which contained the ABA-binding molecule, was unable to suppress the response to SE when added to I-J$^+$ material from either Ly-1 TsiF or the F12 hybridoma. This recapitulates our earlier results which showed that the antigen specificity of these factors is determined by the I-J$^-$ chain.

Genetic Restriction in the Biological Activity of the Hybrid Suppressor Factor Is Determined by the I-J$^+$ Molecule from the F12 Hybridoma. Since molecules from different factors can reconstitute the biological activity of the Ly-1 TsiF, and since the I-J$^-$ hybrid suppressor complex was responsible for antigenic restriction, we asked which molecule was responsible for the genetic restriction exhibited by this hybrid factor. The results in Table III show that the I-J$^+$ molecule from the

TABLE II

I-J$^+$ Chain from ABA-specific TsF$_1$ Can Provide the I-J$^+$ Material Needed for Ly-1 TsiF Activity

Source of antigen-binding chain*	Source of I-J$^+$ material‡	Anti-SE PFC/culture	
		B6AF$_1$	B10.A
—	—	4,600	2,200
A/J (SE)	—	4,100	2,100
	B6	**1,900**	**500**
	A/J	**2,100**	2,900
	F12	**1,200**	2,400
	F3	4,600	2,600
	BW 5147	3,800	2,900
F12 (ABA)	—	5,500	2,800
	A/J	5,000	2,700
	F12	5,100	3,000

* See footnote (*) to Table I. Antigen specificities of factor sources of antigen-binding chains are given in parentheses.

‡ B6 and A/J I-J$^+$ material was obtained from SE-specific Ly-1 TsiF; I-J$^+$ material from F3, F12, and BW 5147 was obtained from the supernatants of these cell lines as described in Materials and Methods.

TABLE III

Genetic Restriction of the Hybrid Suppressor Complex Is Imparted by the F12 I-J$^+$ Chain, Not the SE Antigen-binding Chain

Source of SE-specific antigen-binding chain*	Source of I-J$^+$ material	Anti-SE PFC/culture	
		B6AF$_1$	B10.A
—	—	4,100	7,000
B6	—	3,600	7,900
A/J	—	4,500	8,200
B10.A	—	3,600	6,700
—	A/J	4,600	8,800
B6		**1,200**	6,700
A/J		**1,900**	6,200
B10.A		**700**	6,300
—	F12	4,500	8,600
B6		**1,000**	7,500
A/J		**600**	7,800
B10.A		**700**	6,200
—	B6	3,400	6,200
B6		**700**	**2,700**
A/J		**1,000**	**2,200**
B10.A		**600**	**1,800**

* See footnote (*) to Table I.

TABLE IV

Genetic Restriction Imparted by F12 I-J$^+$ Chain Is Linked to the Igh Gene Complex

Source of SE-specific antigen-binding chain*	Source of I-J material	Anti-SE PFC/culture	
		BALB/c	C.AL20
—	—	2,300	1,000
A/J	—	2,100	1,100
B6	—	2,200	1,200
—	A/J	2,900	1,000
A/J		2,000	**400**
B6		2,000	**100**
—	F12	2,900	1,400
A/J		2,100	**500**
B6		2,800	**100**
—	BALB/c	1,600	800
A/J		**800**	800
B6		**400**	900

* See footnote (*) to Table I.

F12 hybridoma and not the antigen-binding one from the Ly-1 TsiF had to come from cells that genetically matched the assay cells for suppression to be seen. Two important points should be noted here: (*a*) There appears to be no apparent genetic restriction in the ability of the F12 I-J$^+$ molecules to interact with antigen-specific chains to form a functional suppressive complex; and (*b*) the I-J$^+$ molecules from the F12 hybridoma showed a reactivity pattern identical to that of the I-J$^+$ molecule from an A/J Ly-1 TsiF in reconstituting suppressive activity to a number of different antigen-binding chains. We therefore tested whether the activity of the F12 I-J$^+$ molecule was restricted by genes linked to the Igh region using BALB/c (Igh^a) and allotype congenic C.AL20 (Igh^d) mice. The results in Table IV show that, like the I-J$^+$ molecule from A/J Ly-1 TsiF, the hybrid suppressive complex of the SE antigen-binding chain and F12 I-J$^+$ molecule was able to suppress C.AL20 mice, while the response of BALB/c mice was unaffected. These results suggest that the interaction between the hybrid suppressor complex containing the F12 I-J$^+$ chain and its acceptor cell is controlled by genes linked to the Igh gene loci.

Discussion

Network theories involving the role of Igh-linked gene products in the interactions between lymphocytes are based on the idea that antigen-specific molecules carry determinants (idiotypes) which are themselves targets of immune regulation (3). We have previously described (12) an antigen-specific T cell factor that shows an Igh-linked genetic restriction in its ability to induce suppressor cell activity. This allotype-linked genetic restriction is imparted by one portion of the TsF, an I-J$^+$ molecule that does not discernibly interact with antigen (13). The present set of experiments suggests that the Igh-linked genetic restriction exhibited by these factors is not necessarily based on the antigen-recognizing structures found

on these T cell factors. Rather, in the SE system, these antigen-binding structures appear to function by determining the antigen specificity of the suppressive complex. Herein we show that it is the I-J$^+$ antigen-nonspecific molecule that imparts allotype-linked genetic restriction.

The major question raised by these studies is how do these results relate to the earlier findings of idiotype-antiidiotype interactions between T cell subsets? If one wished to maintain a strict network theory of immunoregulation, one would have to hypothesize that the I-J$^+$ molecule is antiidiotypic and sees idiotype on the cell with which it interacts. This explanation is not entirely satisfactory in that the presumed antiidiotypic molecule can come from factors immunized to a number of different antigens (12) and can collaborate with antigen-binding structures with different specificities from any strain to induce suppressor cell function (13). Therefore, if it is antiidiotypic, the idiotypic determinants it recognizes have nothing to do with antigen specificity.

Previous results with ABA-specific suppressor factors (6) have shown that the TsF_1 bears cross-reactive idiotype (CRI)$^+$ determinants that cross-react with some of those found on anti-ABA antibodies from the same strain. The structures are not, however, identical to the major CRI elements found on immunoglobulin with ABA specificity. Passive administration of TsF_1 led to the activation of Ts_2 cells, which bear antiidiotypic determinants and will bind to CRI$^+$ antibody-coated plates (7). We have yet to determine whether our hybrid molecular complex of SE antigen-binding and TsF_1 I-J$^+$ chains induce suppressor cell activity by acting together on a single target cell, and whether the cells induced by this hybrid factor are anti-CRI, as one might predict if the Igh-V-restricting element on the ABA-specific TsF_1 (F12) is indeed CRI. Moreover, we have not yet established if the mechanism of suppression with hybrid molecules is identical to that with conventional TsF. Reciprocal experiments in the ABA system,[2] using F12 I-J$^-$ chain and the I-J$^+$ material from the SE-specific Ly-1 TsiF, have strengthened the contention that the antigen-binding chain imparts antigenic restriction, but the I-J$^+$ chain can be responsible for the Igh-V-linked genetic restriction.

It is clear that our results cannot formally rule out a partial role of a network-type interaction. The data does, however, suggest that the nature of Igh-V-linked genetically restricted interactions may be more complex than previously thought. We suggest that Igh gene products on T cells are homologous to cellular interaction molecules of MHC originally described by Katz and Benacerraf (18). A number of transplantation antigens have been found tightly linked to the Ig heavy chain complex (19, 20) and are most likely the type of molecules that are involved in the allotype-linked, genetically restricted interactions we see with these factors. These allotype-linked restricting elements are found on the I-J$^+$ portion of both the Ly-1 TsiF and the TsF_1, and are not related to the antigen-recognition structures on these T cell factors.

In sum, it appears that the nature of Igh-linked, genetically restricted T cell interactions may depend not on the recognition of unique antigen-binding

[2] Lowy, A., P. M. Flood, A. Tominaga, J. A. Drebin, J. Damrauskas, R. K. Gershon, B. Benacerraf, and M. I. Greene. Analysis of T suppressor factors. Genetic restriction of TsF_1 activity can be imparted by an I-J$^+$ antigen-nonspecific molecule. J. Immunol. In press.

immune receptors by a complementary set of lymphocytes, although this mechanism may play a role in some systems. Rather, Igh restrictions may depend on a much smaller number of determinants, which act as cellular interaction molecules in much the same way as those described for MHC-controlled determinants. The roles of I-J and other antigens in these interactions are currently being investigated.

Summary

Immunized Ly-1 T cells secrete an antigen-specific molecule that will induce Ly-2$^+$ T cells to express suppressive activity. In two separate systems, factors that suppress the primary anti-sheep erythrocyte (SE) plaque-forming cell response of spleen cells in vitro (Ly-1 TsiF) or the contact sensitivity of azobenzenearsonate (ABA)-TsF$_1$ consist of two macromolecules, one which binds antigen and is I-J$^-$, the other which is I-J$^+$ and does not bind antigen. Both of these chains are required for the factor's biological activity. These factors show a genetic restriction in their ability to induce suppression that is linked to the variable region of the Ig heavy chain gene complex (Igh-V). The I-J$^+$ chain from the ABA-specific TsF$_1$ could replace the I-J$^+$ chain needed by the SE-specific Ly-1 TsiF for biological activity. Mixtures of ABA-binding chain with I-J$^+$ material obtained from the SE-specific Ly-1 TsiF had no effect on the primary anti-SE response in vitro. In mixtures of SE antigen-binding chain from Ly-1 TsiF and I-J$^+$ material from the ABA-specific TsF$_1$, it is the I-J$^+$ molecule that determined the factor's Igh-V restriction. Thus, the antigen-combining site of the factor determined the antigen specificity of this factor but is irrelevant to its Igh-V-linked genetic restrictions. The implications of these results for the idiotype network hypothesis are discussed.

We would like to dedicate this manuscript to Dr. Richard Gershon, who, in July of 1983, succumbed in his battle with cancer. The scientific world has suffered a greater loss than it will ever fully appreciate. We, his colleagues, will miss his innovative science, his love for solving life's mysteries, and his warm and endearing personality. Goodbye, Dick.

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