

MOLECULAR COMPOSITION OF AN ANTIGEN-SPECIFIC,  
Ly-1 T SUPPRESSOR INDUCER FACTOR  
One Molecule Binds Antigen and Is I-J<sup>-</sup>;  
Another Is I-J<sup>+</sup>, Does Not Bind Antigen, and Imparts an  
Igh-variable Region-linked Restriction\*

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A number of antigen-specific T cell-produced suppressor factors have been reported to express I-J-controlled antigenic determinants (1, 2). In most cases, the Ly phenotype of the cell producing the biologically active factors has not been determined. One factor, studied by Tada and his associates (2), has been shown to come from Ly-1<sup>-</sup>2<sup>+</sup> cells and to act in combination with an I-J<sup>+</sup> Ly-1,2 cell to amplify Ly-1<sup>-</sup>2<sup>+</sup> suppressor cell activity. Another I-J<sup>+</sup> factor we have described (3) is specified for sheep erythrocytes (SRBC)<sup>1</sup> and is made by Ly-1<sup>+</sup>2<sup>-</sup> I-J<sup>+</sup> cells (Ly-1 TsiF).

The question of how the major histocompatibility complex (MHC) gene product relates to the antigen specificity of the factors activity is not known. In studies of "factors" secreted by immune T cells, no one has as yet succeeded in separating a molecule that expresses an I-J determinant from a molecule that sees antigen. Because an impressive body of circumstantial evidence has suggested that the genes that control the antigen specificity of T suppressor cells are encoded on the 12th chromosome (4) and that the gene that controls the expression of I-J is encoded on the 17th chromosome (5), an important question remains to be answered: How do the two products, I-J and the antigen recognition unit, become associated? Based on the precedent established from studies of better defined antigen-specific products of the immune system (i.e., antibodies), covalent linkage by disulfide bonds would seem to be the most likely answer to this question. Evidence that such may be the case comes from studies of T cell hybridomas that express antigen-specific activity, but the evidence is contradictory.

Taussig and Holliman have shown (6) that an anti-H-2 serum will remove the antigen-binding capacity of biosynthetically labeled material in the supernatant of an SRBC-specific suppressive T hybrid line, but will not do so if the antigen-binding

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<sup>1</sup> *Abbreviations used in this paper:* BRBC, burro erythrocytes; FCS, fetal calf serum; HRBC, horse erythrocytes; Igh, Ig heavy chain complex; Igh-V or VH, Igh variable region; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TsiF, T suppressor inducer factor.

activity comes from NP-40 extracts of the T hybrid line. From this data they infer that the H-2 determinant and the antigen-binding unit are on two separate polypeptides that are linked when the molecule is secreted. The chemical basis for the inferred linkage of the two molecules was not established, but evidence was presented that indicated that the chains were not linked by disulfide bonds.

More recently, Taniguchi and his associates (7) have obtained interesting relevant data with a keyhole limpet hemocyanin (KLH)-specific I-J<sup>+</sup> T cell hybridoma. They have shown that in the secreted form, the hybridomas I-J<sup>+</sup> marker and the antigen-binding moiety travel together on affinity columns, using biological activity as a read-out. These findings are similar to those reported by Benacerraf and co-workers (8). Taniguchi et al. have further shown that extracts of the hybridoma presumably made by ultrasonication can also have their suppressive activity removed by passage through an antigen column or an anti-I-J column. However, in contrast to the secreted material, recombination of the materials that do not bind to KLH with those that do not bind to the anti-I-J serum restores the highly suppressive nature of the extract. Thus, their evidence suggests that there are two distinct molecules in the extract that are synthesized in the cytoplasm of the hybridoma and that do not associate until they are expressed on the cell's surface or secreted from it. Because they have been able to separate the secreted I-J<sup>+</sup> material from the KLH-binding molecule by treatment with 5 mM dithiothreitol, they suggest that the two molecules may be disulfide bonded.

Thus, in two cases using different read-out methods, data have been presented indicating that T cell hybrids make two separate molecules, one that sees antigen and the other that bears H-2 markers, and that these molecules combine in some form of tight association when they are secreted from the cells. In neither of these cases was the Ly phenotype of the cell that fused with the thymoma cell line determined.

Studies with the SRBC-specific I-J<sup>+</sup> Ly-1 T suppressor inducer factor (TsiF) we described yield results somewhat different from any of the previous reported results using I-J<sup>+</sup> antigen-specific T suppressor factors from either normal or tumorous cells. In the supernatants of cultured immune Ly-1 cells, we find two easily disassociated molecules (in fact, they are so easily disassociated that we have no evidence that they are ever associated); one of these is I-J<sup>+</sup> and does not react with antigen, and the other is I-J<sup>-</sup> and binds the SRBC. Neither molecule has biological activity on its own, but biological activity can be achieved by mixing the two separate molecules. We have been able to make "hybrid molecules" by taking I-J<sup>+</sup> material from T suppressor inducer cells of one antigen specificity and mixing them with the antigen-binding material from T suppressor inducer cells of a different specificity. These types of results confirm the separateness of the two molecules required for biological activity and also confirm the observation that the I-J<sup>+</sup> material has no antigen specificity. Because the molecule we are studying will not induce suppression in cells that express Igh variable region (VH) gene-linked polymorphisms that differ from the producer cells, we have been able to use these hybrid molecules to ask the question: Which of the two molecular components used to make the suppressor inducer molecule biologically active imparts the VH-linked restriction? Interestingly and surprisingly, it is the I-J<sup>+</sup> antigen nonbinding molecule that acts as the restricting element. The genotype of the cell that makes the antigen-binding factor appears to be irrelevant; as long as

it is combined with an I-J<sup>+</sup> chain from a mouse expressing the appropriate VH-linked gene, antigen-specific suppressive induction occurs.

### Materials and Methods

*Mice.* C57BL/6 and BALB/c mice, 6–10 wk of age, were obtained from The Jackson Laboratory, Bar Harbor, ME. CB.20, BALB.B, and BC.9 mice were raised in the Yale animal facility with the help of C. A. Janeway, Yale University, New Haven, CT.

*Antigens.* SRBC, horse erythrocytes (HRBC), and burro erythrocytes (BRBC) were obtained from Colorado Serum Company Laboratories, Denver, CO.

*Production and Use of Antisera.* Monoclonal anti-Ly-1.2 and anti-Ly-2.2 were generously supplied by F. W. Shen, Sloan-Kettering Memorial Institute, New York. Anti-I-J<sup>b</sup> serum was prepared by D. B. Murphy by hyperimmunizing B10.A(5R) recipients with a mixture of B10.A(3R) spleen and lymph node cells. Anti-I-J<sup>d</sup> serum was kindly provided by Dr. Chella David. The specificity of the anti-I-J<sup>b</sup> serum was established in two ways: (a) absorption of the serum with B10.A(3R) and not B10.A(5R) removed cytotoxic activity against I-J<sup>b+</sup> cells that participate in the “feedback suppressor circuit” (3); (b) the anti-I-J<sup>b</sup> immunoabsorbents (see below for method of preparation) did not absorb I-J<sup>a</sup> or I-J<sup>d</sup> Ly-1 TsiF. The specificity of the anti-I-J<sup>d</sup> serum was tested only in the second way (e.g., on the immunoabsorbents).

The spleen cells, which were the source of the Ly-1 TsiF, were treated with anti-Ly-2.2 serum in the following manner:  $1 \times 10^7$  spleen cells/ml were incubated in appropriately diluted antisera on ice for 45 min, centrifuged, and then resuspended in a 1:5 dilution of rabbit complement (C') that had been prescreened for low background cytotoxicity. After incubation at 37° for 30 min, the cells were washed twice in balanced salt solution (BSS) and resuspended in tissue culture media that was RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 mM L-glutamine, 25 mM Hepes,  $5 \times 10^{-5}$  M, 2-mercaptoethanol, and antibiotics. The adequacy of the antiserum treatments was established by showing that none of the suppressive factor made in the cultures had Ly-2 TsiF-like activity, i.e., they were not H-2 restricted and could not suppress Ly-2-depleted spleen cells (9).

*Production of the Ly-1-derived Suppressor Inducer Material (Ly-1 TsiF).* Mice were immunized intraperitoneally with 0.2 ml of 20% SRBC twice, at an interval of 2 wk. They were killed 2 wk after the second immunization. A suspension of their spleen cells was treated with anti-Ly-2 and rabbit C' as described above and then cultivated in vitro for 48 h at a concentration of  $1 \times 10^7$  cells/ml of RPMI 1640 supplemented with 10% FCS in a CO<sub>2</sub> incubator. After 48 h of cultivation, supernatant fluids were harvested, centrifuged at 2,500 rpm for 20 min, and passed through millipore filters. Although no antigen was added to the factor(s) producing cells during the 48-h incubation period, it is possible that small amounts of residual antigen from the previous immunizations were carried over into the culture. The need for antigen induction in vitro to get factor production is presently under investigation. Previous studies (3) have shown that Ly-1 cells actually produced the factor by the use of cells that were selected on an anti-Ly-1-coated dish and were 95–98% Ly-1<sup>+</sup>, as judged by immunofluorescence as a source of factor.

*Absorption of Soluble Factor.* For absorption with RBC, 1 ml of supernatant material was mixed with 0.1 ml of a 50% suspension of erythrocytes for 1 h on ice and then centrifuged. For other absorptions, supernatants were passed over immunoabsorbents prepared by conjugation of antisera to cyanogen bromide-activated Sepharose using the method of Axen et al. (10). We appreciate the help of G. Michael Iverson in preparing these columns.

*In Vitro Primary Anti-RBC Response.* A modification of the cell culture technique described initially by Mishell and Dutton (11) was used to generate in vitro primary anti-RBC (sheep and horse) response. Unprimed spleen cells were cultured with a concentration of  $5 \times 10^6$  cells/ml of culture media in Linbro (Linbro Chemical Co., Hamden, CT.) 24 flat-bottomed dishes at 37°C in a 5% CO<sub>2</sub> incubator with 0.1 ml of 0.4% RBC for 5 d. Plaque-forming cell (PFC) responses were determined by Cunningham's modification of the Jerne plaque assay (12). The mean and standard error of PFC were calculated from triplicate cultures. The various Ly-1 TsiF materials were added to assay cultures at the time of initiation at a final dilution of 1:10. When recombination of separated macromolecules that compose the Ly-1 TsiF were done, each macromolecule was used at a final dilution of 1:20, i.e., there was a 50:50 reconstitution.

## Results

*Separation of the Antigen-binding and the I-J<sup>+</sup> Molecules by Absorption Studies.* The supernatants from the 48-h cultures of immunized Ly-1 cells (Ly-1 TsiF) were fractionated by their antigen-binding capacity or by their ability to stick to anti-I-J immunoabsorbent columns. Neither the filtrate coming through an I-J column, nor the eluate from that column, nor the material left after antigen absorption, had any suppressive capacity on the primary response to SRBC in Mishell-Dutton cultures (Table I). Recombination experiments showed that both the filtrate from the I-J column and the material left after antigen absorption could reconstitute suppressive activity when added to the I-J eluate; however, mixing the antigen filtrate with the I-J eluate did not reconstitute activity. Thus, the material eluted from the I-J column and the material left after antigen absorption displayed similar properties, whereas the material eluted from the I-J column was different. The most straightforward interpretation of these data is that the I-J eluate and the antigen filtrate contained the same molecule that did not have the capacity to recognize antigen. The I-J filtrate contained a separate molecule that could see antigen, and both molecules were required for the expression of biological activity.

We did two types of experiments to test this conclusion; in one set of experiments we absorbed the filtrate and the eluate with specific antigen to see which molecule recognized the antigen; the results of such an experiment (Table II) show that antigen can absorb all the biological activity from the I-J filtrate but not the I-J eluate. Thus, this experiment confirms two of the conclusions drawn from the experimental data in Table I: (a) two separate molecules are required for biological activity, and (b) the I-J<sup>-</sup> molecule is antigen-specific and the I-J<sup>+</sup> molecule is not.

*Separation of the Molecule That Confers Antigen Specificity from the I-J<sup>+</sup> Molecules by Doing Factor Reconstitution Studies.* To further test the conclusions drawn from absorption

TABLE I  
*Biological Activity of Ly-1 TsiF Is Dependent on Two Separate Molecules*

Source of Ly-1 TsiF			B6 spleen cells		Comments
I-J filtrate*	I-J eluate*	Antigen filtrate*	Experiment 1	Experiment 2	
<i>PFC/culture</i>					
-	-	-	1,900	2,300	Control response
+	-	-	1,900	2,700	No suppression: all three treatments inactivate factor
-	+	-	1,600	2,200	
-	-	+	2,300	ND‡	
+	+	-	600	400	Suppression: I-J eluate or antigen filtrate can give I-J filtrate suppressive activity
+	-	+	800	ND	
-	+	+	2,100	ND	No suppression: I-J eluate and antigen filtrate do not reconstitute suppression

The conclusions are as follows. I-J eluate and antigen filtrate are molecule 1. I-J filtrate is molecule 2. Molecule 1 is nonantigen binding; molecule 2 binds antigen.

\* See Materials and Methods for details of how these separation techniques were performed and for dose of material added to cultures.

‡ Not done.

TABLE II  
*I-J<sup>+</sup> Molecule in Ly-1 TsiF Molecular Complex Does Not Bind Antigen; the I-J<sup>-</sup> Molecule Does (Absorption)*

Source of Ly-1 TsiF		B6 spleen cells
I-J filtrate*	I-J eluate*	
		<i>PFC/culture</i>
-	-	1,100
+	-	1,100
-	+	900
+	+	300
+ (antigen absorbed)‡	+	1,000
+	+ (antigen absorbed)‡	300

\* See Materials and Methods for details of how these separation techniques were performed and for dose of material added to cultures.

‡ Absorbed with SRBC (see Materials and Methods).

TABLE III  
*I-J Molecule in Ly-1 TsiF Molecular Complex Does Not Have Antigen Specificity; the I-J<sup>-</sup> Molecule Does (Reconstitution)*

Source of Ly-1 TsiF		B6 spleen cells	
I-J filtrate*	I-J eluate*	SRBC‡	HRBC§
			<i>PFC/culture</i>
-	-	1,200	1,800
SRBC	SRBC	700	2,200
SRBC	HRBC	450	3,100
HRBC	HRBC	ND¶	800
HRBC	SRBC	1700	800

\* See footnote, Table I.

‡ Primary in vitro response to SRBC.

§ Primary in vitro response to HRBC.

|| Antigen used to immunize factor producing mice.

¶ Not done.

studies, we did a series of experiments making hybrid molecules in which we mixed the I-J filtrate and the I-J eluate of Ly-1 TsiF factors that were specific for different heterologous erythrocytes. The results of two of these types of experiments are presented in Tables III and IV. The experiment shown in Table III used Ly-1 TsiF from SRBC and HRBC-immune Ly-1 cells. The results show that the specificity of the factors comes from the material in the I-J filtrate; the material in the I-J eluate will reconstitute the specific suppressive activity of both SRBC and HRBC antigen-binding molecules with equal efficiency. The results in Table IV come from the same type of experiment using BRBC cells in place of HRBC cells and yields the same type of data. That is, (a) the I-J eluate has no antigen specificity, (b) it can reconstitute biological activity of I-J filtrates, and (c) such reconstituted factors express the specificity of the I-J filtrate (i.e., the molecule that determines antigen specificity is I-J<sup>-</sup>).

TABLE IV  
*I-J<sup>+</sup> Molecule in Ly-1 TsiF Molecular Complex Does Not Have Antigen Specificity; the I-J<sup>-</sup> Molecule Does (Reconstitution)*

Source of Ly-1 TsiF		B6 spleen cells		
I-J filtrate*	I-J eluate*	Experiment 1		Experiment 2
		SRBC‡	BRBC§	SRBC‡
—	—	2,900	1,400	1,400
		<i>PFC/culture</i>		
SRBC	SRBC	700	1,200	400
SRBC	BRBC	700	1,300	350
BRBC	BRBC	2,700	500	1,300
BRBC	SRBC	1,900	540	1,000

\* See footnote, Table I.

‡ Primary in vitro response to SRBC.

§ Primary in vitro response to HRBC.

|| Antigen used to immunize factor producing mice.

*The I-J<sup>+</sup> Molecule That Does Not Bind Antigen Is Responsible for the VH Restriction of the Factor's Biological Activity.* Having shown that molecules from different factors can reconstitute biological activity and that the molecule that is I-J<sup>-</sup> imparts antigen specificity, we were now able to ask the important question: Which of the two molecules was responsible for the VH-linked restriction that the intact Ly-1 TsiF suppressor molecule showed? The results of a series of experiments using immunoglobulin (Igh) and H-2 congenic mice to supply one of the two necessary molecules (Table V) showed that the I-J<sup>+</sup> molecule and not the antigen-binding one had to come from cells that expressed the same polymorphisms in the Igh complex as did the assay cells for suppression to be seen. The easiest way to decipher the data that allows this conclusion is to note that in all cases where the cellular source of the I-J<sup>+</sup> molecule is identical at the Igh complex with the B6 acceptor cells (identified by a + marker in the appropriate Ig column; the one with an asterisk) there is significant suppression except for the control experiment in which there was no antigen-binding molecule because of antigen absorption (experiment 5). Note the contrast in experiment 5, where antigen absorption of the I-J eluate did not remove suppressive activity. This finding indicates that no contaminant antigen-binding material was present in the reconstituting I-J<sup>+</sup> eluate. In all other columns, one can note that suppression takes place when (-) markers are present, showing the irrelevancy of (a) H-2 haplotype in either molecule and (b) Ig haplotype in the antigen-binding molecule.

### Discussion

The notion of interactions between products of Ig gene complexes forms the foundation on which network theories are built (13). It has been presumed that the antigen-specific molecules carry the determinants that are involved in idiotype anti-idiotype regulatory reactions. Our results show that at least in one case this is not true. There is an immunoregulatory T cell factor (3) that contains a distinct molecule that interacts with VH gene-controlled products and that does not see antigen (the

TABLE V  
*Antigen Nonbinding I-J<sup>+</sup> Molecule Contains Igh- (or Anti-Igh)-linked Cell Interaction Molecule; Antigen-binding Molecule Not Involved in Igh-restricted Interaction (Studies with BAB.14 Mice Map the Igh Restriction to Igh-V [3])*

Experiment number	Source of antigen binding molecule*			Source of I-J <sup>+</sup> molecule			B6 spleen cells	Suppression
	Mouse strain	Identity with assay cells		Mouse strain	Identity with assay cells			
		Ig‡	H-2‡		Ig‡§	H-2‡		
							<i>PFC/culture</i>	
1	None			None			1,400	Standard
	None			B6	0	0	1,200	-
	B6	+	+	B6	+	+	400	+
	BALB.B¶	-	+	B6	+	+	300	+
2	None			None			2,800	Standard
	None			CB20**	0	0	4,000	-
	CB20	+	-	CB20	+	-	900	+
	CB20	+	-	BALB/c‡‡	-	-	3,200	-
	BALB/c	-	-	CB20	+	-	400	+
3	None			None			1,900	Standard
	B6	+	+	BALB.B	-	+	2,200	-
	BALB.B	-	+	B6	+	+	400	+
4	None			None			1,900	Standard
	B6	+	+	BC.9§§	-	+	2,300	-
	BC.9	-	+	B6	+	+	800	+
5	None			None			4,200	Standard
	B6	+	+	BC.9	-	+	3,200	-
	BC.9	-	+	B6	+	+	1,000	+
	BC.9	-	+	B6	+	+	4,700	-¶¶
	BC.9	-	+	B6	+	+	1,400	+

\* See footnote, Table I.

‡ (+), homology with B6 assay cells; (-), no homology; (0), no antigen-binding chain added (i.e., control).

§ This column identifies key molecule that imparts VH restriction.

|| H-2<sup>b</sup>; Ig<sup>b</sup>.

¶ H-2<sup>b</sup>; Ig<sup>a</sup>.

\*\* H-2<sup>d</sup>; Ig<sup>b</sup>.

‡‡ H-2<sup>d</sup>; Ig<sup>a</sup>.

§§ H-2<sup>b</sup>; Ig<sup>a</sup>.

||| Absorbed with SRBC (see Materials and Methods).

¶¶ No suppression due to lack of antigen-binding molecule. (0), no antigen-binding chain added (i.e., control).

genetic mapping done in the studies presented in this paper does not distinguish between VH- and CH-controlled interactions, but previous studies (3) with this factor, using the crossover in BAB.14 mouse, have shown that the relevant structures are controlled by VH). If one wishes to maintain a strict network theory of immunoregulation, one could hypothesize that the molecule that fails to see antigen is anti-

idiotype and thus sees idiotype on the cell with which it interacts. (Because the molecule does not see antigen, it would be like the Ab3 (anti-anti-idiotypic) in network terminology [14]), with one major exception: this particular Ab3 bears a marker controlled by genes on the 17th chromosome (e.g., I-J).

However, because the factor has no biological activity without an accompanying molecule that does see antigen, a role for the antigen-recognizing molecule must be found. If the function of the antigen-binding molecule is to focus the factor on the appropriate target cell via an antigen bridge (which seems likely), then the tables are turned, and a novel role for the I-J<sup>+</sup> anti-idiotypic molecule must be found. It is possible that two focusing events are required to bring the two molecules to the appropriate cells, and then biological function is brought about by an interaction between the two molecules on the cell surface, perhaps in a way similar to the activation of zymogens by proteases. Alternatively, the two molecules may not come together but may act on different cells. Whatever roles the two molecules play, the importance of the findings is that it gives equality if not primacy to antigen-recognizing molecules (as opposed to "Igh-encoded idiotype-recognizing antibodies") in immune regulation.

It is clear that our studies cannot formally rule out a partial role of a network type interaction, as there is sufficient plasticity in the theory that would allow it to accommodate our findings using either the *ad hoc* assumptions mentioned above or others. However, we find the data so similar to that generated in studies (15) of T cell dual recognition, where MHC-controlled determinants are the restricting elements, to make us favor an alternate explanation for the data. We suggest that domains of the antigen-binding units on some T cells (or on closely associated structures) express gene products that are homologous to some products of the MHC and act as classical cell interaction structures as originally enunciated by Katz and Benacerraf (16). Interestingly, the cell interaction structures they defined act as potent transplantation antigens. It should therefore be recalled that transplantation antigens have been found (by genetic backcross studies) to be tightly linked to the Ig heavy chain complex (17, 18). Our guess would be that these are the type molecules that are involved in the VH anti-VH interactions and that are required for biological activity of the factor we have described. If this is true and we can show that both the antigen binding and the I-J<sup>+</sup> molecule act on the same cell, then our model would unambiguously show a case where dual recognition is required for biological activity. Altered self recognition on its own cannot explain our results because the specific antigen-binding molecule is absolutely required for biological function.

The intriguing question that remains to be answered is: Why is a product controlled by a gene on the 17th chromosome (I-J) a marker for a molecule that interacts with another molecule controlled by a gene product on the 12th chromosome (VH)? Standard adaptive differentiation theories of VH-encoded products cannot be taking place in the system we have described because the H-2 haplotype of the I-J<sup>+</sup> product is irrelevant in the cell interaction. Thus, VH cannot have learned to see an H-2 haplotype that was not present during the cell's differentiative history. The same line of logic can be used to say that our results are unlikely to be due to the presence of contaminating anti-(anti-I-J) antibodies in our anti-I-J sera. Such antibodies would be influenced by the I-J haplotypes of the immunizing and/or antibody-producing cells and thus would show an MHC linkage.

It is possible that some product of the 17th chromosome marked by the I-J determinant can adaptively differentiate and become VH restricted. Although a short while ago this notion might have seemed far fetched at best, as we learn how little we know about gene rearrangement mechanisms (19), the possibility that products of one chromosome influence the expression of structural genes on chromosomes other than those known to contain variable regions (i.e., Ig loci) becomes less remote.

A more conventional explanation of our findings would be that I-J encodes a glycosyl transferase and that we are looking at a 12th chromosome gene product with a carbohydrate determined specificity given to it by an enzyme encoded on the 17th chromosome.

The most conventional explanation for the data (and also the most violative of Occam's Razor i.e., do not create more entities [read molecules] than are necessary for the explanation of a phenomenon) is that the I-J<sup>+</sup> VH-restricting molecule is made of two separate polypeptides, and the biological function of the Ly-1 TsiF is thus dependent upon three separately encoded molecules (one that sees antigen; one that gives VH restriction; and one that is I-J<sup>+</sup> and of which we do not know what its function is or might be) that interact either directly or indirectly with one another.

Lastly, we should comment on why we can mix an I-J filtrate with an I-J eluate from a noncellular extracted biologically active product and achieve activity, whereas other workers cannot. The simplest answer would be that our product is not a secreted one but is released by dying cells in culture. This would then make our results compatible with those of workers studying T cell hybridomas (6, 7), and thus there would be no contradictory evidence between laboratories (except for the differences in the finding of covalent linkage of the molecules in secreted factors by Taniguchi et al.). It should, however, be kept in mind that the material we are studying comes from Ly-1 cells and shows VH-linked restriction. None of the other studies on I-J<sup>+</sup> material have shown that the material being studied comes from this T cell subset or shows this restriction. It is well known that other T cell subsets make I-J<sup>+</sup> molecules, and thus the lack of molecular association of secreted products may be dependent upon the nature of the cell that is making the product. In any case, the answer to this question is far less important than are those of how the I-J<sup>+</sup> material gives VH restriction and what the chemical nature of the VH-linked cell interaction structures is. Our present work is focused on answering these questions.

### Summary

Immunized Ly-1<sup>+</sup>2<sup>-</sup> T cells (Ly-1 cells) make an antigen-specific soluble suppressor product (Ly-1 TsiF) that will induce Ly-2<sup>+</sup> cells to express suppressive activity but only if the Ly-2<sup>+</sup> cells and the Ly-1 producer cell share genetic polymorphisms that are linked to the Igh locus and in particular that part where the Igh-V (or VH) is encoded. Ly-1 TsiF can be separated into two entities, one binds antigen and does not express I-J determinants, and the other is I-J<sup>+</sup> and does not bind antigen. Neither of these "subfactors" has biological activity, but a 50:50 mixture of them reconstitutes biological activity that expresses the antigen specificity of the antigen-binding molecule. Any of the three heterologous erythrocytes (antigens) studied can be used for immunization to produce the I-J<sup>+</sup> nonantigen-binding factor, i.e., the I-J<sup>+</sup> moiety makes no contribution to the factor's specificity. It does, however, determine the intact factor's Igh-V linked restriction. Thus, the antigen combining site of the factor

is irrelevant to the factor's Igh-V restriction but crucial for its specificity. The I-J<sup>+</sup> molecule does not bind antigen nor influence the factor's antigen specificity but expresses the Igh-V polymorphism (or anti-Igh-V polymorphism) that is required for the transmission of an inductive signal to the factor's Ly-2<sup>+</sup> acceptor cell.

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