PROPERTIES OF THE ANTIGEN-SPECIFIC SUPPRESSIVE T-CELL FACTOR IN THE REGULATION OF ANTIBODY RESPONSE OF THE MOUSE*

III. Dual Gene Control of the T-Cell-Mediated Suppression of the Antibody Response

By MASARU TANIGUCHI, TOMIO TADA, and TAKESHI TOKUHISA

(From The Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan 280)

Our previous studies demonstrated an antigen-specific T-cell factor that can suppress primary and secondary IgG antibody response of mice in both in vivo and in vitro experimental conditions (1, 2). The factor was extractable from thymocytes and spleen cells of mice that had been immunized with a relatively high dose of carrier antigens, and exerted a suppressive effect on the antibody response against a hapten coupled to the same carrier. The specificity of this factor was firmly established by its binding affinity for the antigen, although no immunoglobulin determinants were detectable by absorption studies using various anti-immunoglobulin antisera. The molecular weight of the T-cell factor was between 35,000 and 55,000 daltons, the value being much lower than those of immunoglobulins. Available evidence indicated that the factor suppresses the helper T cell having the same carrier specificity for the antigen rather than the hapten-specific B cell.

Further studies demonstrated that the T-cell factor is a product of a gene or genes present in the major histocompatibility complex. It has been shown that the suppressive activity was consistently removed by absorption with alloantisera reactive with the products of genes in the I region of the H-2 complex, more exactly in the I-A and/or I-B subregions (2, 3). In keeping with these findings, it has also been shown that the T-cell factor derived from one strain of mice can only suppress the response of syngeneic and H-2 histocompatible mouse strains, indicating that identities among genes in the H-2 complex between the suppressor T cells and their target cells are required for effective suppression (1, 3). The most feasible explanation for this histocompatibility requirement is that the acceptor site of the target cells to the suppressive T-cell factor is also determined by a gene or genes in the H-2 complex, and thus a complementation of paired genes in the H-2 complex is required for the suppressive cell interaction. The present investigation was undertaken to determine whether such a two-gene model is, in fact, applicable to the suppressive cell interactions, and to further determine the target cells expressing such acceptor sites.

Materials and Methods

Animals. BALB/cAnN, CBA, C3H/He, A/J, C57BL/6J, SJL, and their F_1 hybrids were produced in our animal facilities. Congeneic strains of B10 background were also raised in our animal facility. Breeding pairs of B10, B10.BR, B10.D2, and B10.A were kindly provided by Dr. K. Moriwaki of the National Institute of Genetics, Mishima, Japan. B10.S are the gift of Dr. C. S. David of the Department of Genetics, Washington University School of Medicine, St. Louis, Mo.

Antigens. Keyhole limpet hemocyanin (KLH)[1] was purchased from Calbiochem, San Diego, Calif. Dinitrophenylated KLH (DNP_{770}-KLH) was prepared by coupling with DNP-sulfonic acid according to the method by Eisen et al. (4).

Immunization of Animals. To prepare the suppressive T-cell factor, animals were immunized with two intraperitoneal injections of 100 μg of soluble KLH without adjuvant at a 2-wk interval. They were killed 2 wk after the second immunization, and their thymuses and spleens were processed as below.

To obtain DNP-KLH-primed spleen cells, mice were primed with 100 μg of DNP-KLH with 1 × 10^9 *Bordetella pertussis* vaccine. They were killed by exsanguination 4 wk after the priming, and their spleen cells were harvested.

Preparation of the Suppressive T-Cell Factor. The thymuses and spleens of KLH-primed mice were placed in a small quantity of chilled Eagle's minimal essential medium (MEM). They were minced by forceps and then gently pressed between two glass slides to release the cells. The cells were washed three times with cold MEM, and were suspended in a small quantity of MEM to make the suspension of 5 × 10^8/ml. The suspension was then subjected to sonication for 2 min in ice with a Tomy UR-150P Sonicator, Tomy Seiko Co. Ltd., Tokyo, Japan. The cell-free supernate was obtained by ultracentrifugation at 40,000 g for 1 h. In most of our present experiments, thymocyte extract (T extract) was used to achieve in vitro suppression of the antibody response.

Cell Culture Technique. The modified Marbrook culture system was utilized to induce hapten-specific in vitro secondary response of primed spleen cells as described in the previous paper (2). In brief, mice were primed with an intraperitoneal injection of 100 μg of DNP-KLH mixed with 10^9 *B. pertussis* vaccine. 4 wk after the immunization, 10^7 of primed spleen cells were cultured with 0.1 μg of DNP-KLH in MEM enriched with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) in Marbrook culture system (5). The culture was maintained at 37°C in an atmosphere of 10% of CO_2 in air for 5 days. The suppressive T extract at a dose comparable to 10^7 original thymocytes was added at the beginning of the culture. After a 5-day culture, the numbers of DNP-specific IgG plaque-forming cells (PFC) were assayed using sheep erythrocytes coupled with DNP_{31}-bovine serum albumin as described previously (2).

Results

Requirement of Histocompatibility for Effective Suppression. Our previous study indicated that the suppressive T-cell factor of BALB/c strain could not suppress the antibody response of histoincompatible C57BL/6J mice (and vice versa) in in vivo experiments (1). Therefore, we have attempted to study this histocompatibility requirement using various combinations of mouse strains and their F_1 hybrids as the donors and recipients of the suppressive T-cell factor. KLH-specific T-cell factor was obtained from BALB/c, C3H, CBA, and (BALB/c × CBA)F_1, and was added to the culture of DNP-KLH-primed spleen cells of various strains of mice. The combinations of the donor and recipient are shown in Table I. The numbers of indirect (IgG) PFC after a 5-day culture with and without the suppressive T-cell factor were compared.

As may be inferred from Table I, the suppressive T-cell factor from one strain

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[1] *Abbreviations used in this paper:* C, guinea pig complement; DNP, 2,4-dinitrophenyl; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; PFC, plaque-forming cell; T extract, thymocyte extract.

TABLE I

Requirement of the Identities among Genes in the K and I Regions of H-2 Complex for the Effective Suppression

Donor of T extract	Responding spleen cells	Identities of H-2 subregions	Anti-DNP IgG PFC/culture		Suppression
			Control	with T extract	
					%
BALB/c	BALB/c	K, I, S, D	3,251 ± 148	447 ± 158	86
	C3H	None	2,557 ± 207	2,701 ± 62	0
	CBA	None	1,534 ± 58	1,325 ± 206	0
	A/J	S, D	4,672 ± 490	4,531 ± 448	0
	(BALB/c × CBA)F₁	K, I, S, D	1,257 ± 24	98 ± 84	92
	(BALB/c × A/J)F₁	K, I, S, D	3,285 ± 101	829 ± 171	75
CBA	CBA	K, I, S, D	1,160 ± 144	81 ± 19	93
	C3H	K, I, S, D	1,169 ± 104	224 ± 36	81
	A/J	K, I	2,186 ± 739	565 ± 132	74
	BALB/c	None	2,667 ± 108	2,989 ± 227	0
	(BALB/c × CBA)F₁	K, I, S, D	1,103 ± 105	32 ± 20	97
	(BALB/c × A/J)F₁	K, I	806 ± 130	50 ± 10	94
C3H	C3H	K, I, S, D	1,208 ± 99	340 ± 102	72
	CBA	K, I, S, D	1,632 ± 47	406 ± 131	75
	A/J	K, I	4,246 ± 150	1,850 ± 487	56
	BALB/c	None	1,241 ± 190	1,192 ± 145	0
	(BALB/c × CBA)F₁	K, I, S, D	1,257 ± 24	191 ± 35	85
	(BALB/c × A/J)F₁	K, I, S, D	2,065 ± 277	1,041 ± 291	56
(BALB/c × CBA)F₁	(BALB/c × CBA)F₁	K, I, S, D	2,229 ± 65	190 ± 52	91
	BALB/c	K, I, S, D	2,045 ± 200	243 ± 74	88
	CBA	K, I, S, D	2,156 ± 125	590 ± 170	73
	C3H	K, I, S, D	1,877 ± 210	103 ± 25	95
	A/J	K, I	4,232 ± 361	815 ± 342	76
	SJL	None	4,337 ± 187	4,484 ± 404	0

of mice can greatly suppress the responses of H-2 histocompatible strains. Thus the BALB/c factor can suppress the BALB/c response, but not C3H, CBA, and A/J responses. Similarly the H-2^k factors derived from CBA and C3H mice could equally suppress both CBA and C3H responses, while failing to suppress the response of BALB/c having different H-2 haplotype. The T-cell factor obtained from (BALB/c × CBA)F₁ could suppress the response of syngeneic F₁ as well as those of both parental strains. Therefore, the suppressor molecules reactive to both parental strains are codominantly expressed in F₁ suppressor T cells. It was also shown that the responses of (BALB/c × CBA)F₁ and (BALB/c × A/J)F₁ cells were suppressible either by H-2^d or H-2^k factors, indicating that the acceptor sites for both factors are codominantly expressed on F₁ spleen cells.

An important point in this experiment is that the factors from CBA and C3H $(H$-$2^k)$ mice but not BALB/c $(H$-$2^d)$ mice can suppress the response of A/J $(H$-$2^a)$ mice which share the same K and I regions with H-2^k strains. Also, the H-2^k factor can suppress the response of (BALB/c × A/J)F₁ mice. Therefore, it is clear that the identities among genes in the K and I regions of the H-2 complex between the donor and recipient are both necessary and sufficient for the effective suppression in cross-strain experiments. Definitely no suppression was observed in any combinations of H-2 histoincompatible strains so far tested.

Failure to Produce the Suppressive T-Cell Factor in A/J Strain.   As mentioned previously, the T-cell factor from H-2^k mice can suppress the response of A/J mice, implying that spleen cells of A/J mice have acceptor sites for the H-2^k

factor. However, the T extract obtained from A/J mice consistently failed to suppress A/J, CBA, and C3H responses as depicted in Table II. We have confirmed several times this peculiar behavior of A/J mice with different antigen doses and immunization schedules.

Even more peculiar is the genetic trait of this inability to produce the suppressive T-cell factor. When (BALB/c × A/J)F_1 was used as the donor, the T-cell factor from this F_1 could suppress responses of both (BALB/c × A/J)F_1 and BALB/c, but not the response of the other parental partner A/J. The F_1 factor also failed to suppress the responses of H-2^k mice (CBA and C3H). Therefore, the production of the suppressive T-cell factor per se is genetically dominant, but the produced factor is strictly strain-specific, and thus the inability to produce the H-2^k reactive factor of A/J mice is also inherited by the F_1 mice (Table II).

Inability to Express the Acceptor Site for the Suppressive T-Cell Factor in B10 Congeneic Strains. Quite contrary to A/J strain, several congeneic strains of B10 background were shown to lack the expression of acceptor site for the H-2 compatible suppressive T-cell factor, while being able to produce the factor itself. The T-cell extracts were obtained from B10, B10.D2, B10.BR, B10.A, and B10.S strains, and were tested in syngeneic and H-2 histocompatible target cells. As shown in Table III, the extracts from B10 congeneic strains did not suppress the responses of syngeneic spleen cells, whereas the same extract could suppress the responses of H-2 histocompatible partners of non-B10 background. Similarly, the extracts derived from non-B10 background, despite having the definite suppressive activity in syngeneic combinations, could not suppress the responses of H-2 identical B10 congeneic strains. Thus for example, the response of B10.S spleen cells was not suppressed by either syngeneic B10.S or the H-2 identical SJL T-cell factor. On the other hand, both the T-cell extracts of B10.S and SJL could suppress the response of SJL only, but not the B10.S response. Furthermore, in some cases a definite enhancement was observed in the response of B10 congeneic strains given the T-cell extract which had been shown to be inhibitory in H-2 identical partners. From this series of experiments, it was concluded that congeneic strains of B10 background can produce the suppressive T-cell factor which suppresses the response of other H-2 histocompatible spleen cells, but are incapable of accepting the suppressive T-cell factor from either syngeneic or H-2 identical strains. This conclusion will be reinforced by the failure of absorption of the T-cell factor by spleen cells of B10 congeneic mice as will be shown below.

The Genetic Trait of the Suppressor-Acceptor Expressions. Since A/J and B10.A both having the same H-2 haplotype lack one of the expressions of the suppressor or acceptor, we crossed these strains to obtain their F_1 hybrid. The ability of the (A/J × B10.A)F_1 to produce the suppressive T-cell factor as well as to accept the factor was tested by combinations of F_1 and parents as the donor and recipient.

As shown in Table IV, the (A/J × B10.A)F_1 could produce the T-cell factor which can suppress both A/J and F_1 responses. The same factor could not suppress the response of the other parental strain B10.A which lacks the acceptor site. Similarly, the response of (A/J × B10.A)F_1 is suppressible by the factors derived from F_1 and B10.A but not from A/J. Thus both the suppressor and acceptor are dominantly expressed on the cells of F_1 whose parents lack

TABLE II

Failure to Produce the Suppressive T-Cell Factor in A/J Mice

Donor of T extract	Responding spleen cells	Identities of H-2 subregions	Anti-DNP IgG PFC/culture		Suppression
			Control	with T extract	
					%
A/J	A/J	K, I, S, D	4,976 ± 248	4,935 ± 264	0
	C3H	K, I	4,503 ± 365	4,358 ± 376	0
	CBA	K, I	2,556 ± 109	2,700 ± 212	0
	BALB/c	S, D	3,353 ± 110	3,799 ± 128	0
	(BALB/c × A/J)F₁	K, I, S, D	3,076 ± 125	3,077 ± 332	0
(BALB/c × A/J)F₁	(BALB/c × A/J)F₁	K, I, S, D	5,393 ± 349	1,651 ± 329	70
	BALB/c	K, I, S, D	3,251 ± 148	1,012 ± 135	70
	A/J	K, I	3,143 ± 100	3,166 ± 288	0
	C3H	K, I	2,557 ± 207	2,969 ± 531	0
	CBA	K, I	1,534 ± 58	1,524 ± 68	0

either one of these expressions.

Presence of an Acceptor Site for the Suppressive T-Cell Factor on T Cells but not on B Cells and Macrophages. The results stated above indicate that only H-2 histocompatible partner can accept the suppressive T-cell factor of other strains, and such an acceptor site is lacking in certain strains. We have already presented an indirect evidence in a previous publication (2) that the target of the suppressive T-cell factor is the T cell but not B cell. This was based on the fact that the suppressive T-cell factor did not suppress the in vitro antibody response unless the helper T cell having an identical specificity to the suppressive T-cell factor coexisted. The result suggested that the factor does not act directly on B cells, but may suppress the helper T cell resulting in the overall reduction of antibody response.

To obtain more direct evidence for the presence of acceptor sites on T cells, the suppressive T extract was absorbed with various cell types from normal syngeneic or allogeneic mice. Suspensions of thymocytes, spleen cells, and bone marrow cells were obtained from normal C3H and BALB/c mice. A fraction of non-θ-bearing cells was obtained by treatment of spleen cells with anti-θ and complement. Macrophages were obtained as the glass-adherent peritoneal exudate cells. An aliquot of the suppressive T-cell extract corresponding to 5×10^7 thymocytes was incubated with 1 to 2×10^8 of each cell type at 4°C for 1 h with constant agitation. The cells were spun down, the supernate was sterilized by passing through a millipore membrane filter, and the residual suppressive activity was assayed by adding the supernate to the culture of primed spleen cells.

Table V demonstrates that the suppressive activity of the T extract can be removed by incubation with syngeneic thymocytes and spleen cells, but not with anti-θ-treated spleen cells, peritoneal macrophages, or bone marrow cells in both C3H and BALB/c mice. It was noted that a larger number of thymocytes was always required for the complete removal of the suppressive activity, suggesting that only a certain proportion of thymocytes expresses the acceptor site or that the density of the acceptor site on thymocytes is lower than that on spleen cells. The cells expressing the acceptor site in spleen cells are anti-θ sensitive. These results infer that the acceptor site for the suppressor molecule

TABLE III

Lacking of the Acceptor Site for the Histocompatible Suppressive T-Cell Factor in Congeneic Strains of B10 Background

Donor of T extract	Responding spleen cells	Identities of *H-2* subregions	Anti-DNP IgG PFC/culture		Suppression
			Control	with T extract	
					%
B10	B10	*K, I, S, D*	4,357 ± 324	4,497 ± 405	0
	C57BL/6	*K, I, S, D*	1,163 ± 209	368 ± 120	68
C57BL/6	C57BL/6	*K, I, S, D*	1,163 ± 209	393 ± 150	66
	B10	*K, I, S, D*	4,357 ± 324	5,047 ± 546	0*
B10.D2	B10.D2	*K, I, S, D*	5,268 ± 98	5,655 ± 388	0
	BALB/c	*K, I, S, D*	1,047 ± 65	412 ± 48	61
	B10.BR	None	951 ± 86	987 ± 82	0
	C3H	None	1,424 ± 64	1,699 ± 197	0
BALB/c	BALB/c	*K, I, S, D*	1,047 ± 65	75 ± 27	93
	B10.D2	*K, I, S, D*	5,268 ± 98	5,373 ± 194	0
B10.BR	B10.BR	*K, I, S, D*	1,459 ± 68	1,422 ± 90	0
	C3H	*K, I, S, D*	3,552 ± 197	1,106 ± 151	65
C3H	C3H	*K, I, S, D*	3,552 ± 197	1,589 ± 316	55
	B10.BR	*K, I, S, D*	1,459 ± 68	1,488 ± 77	0
	A/J	*K, I*	4,246 ± 150	1,850 ± 487	56
	B10.A	*K, I*	5,436 ± 92	5,426 ± 236	0
B10.A	B10.A	*K, I, S, D*	5,436 ± 92	5,295 ± 329	0
	A/J	*K, I, S, D*	3,333 ± 182	1,581 ± 270	53
	B10.BR	*K, I*	1,367 ± 231	1,415 ± 42	0
	C3H	*K, I*	3,574 ± 197	1,124 ± 225	69
B10.S	B10.S	*K, I, S, D*	3,869 ± 27	6,347 ± 435	0*
	SJL	*K, I, S, D*	4,337 ± 187	1,595 ± 47	63
SJL	SJL	*K, I, S, D*	4,337 ± 187	1,198 ± 145	72
	B10.S	*K, I, S, D*	3,869 ± 27	3,599 ± 176	0

* Enhancement.

is, in fact, present on the surface of a T-cell subpopulation but not on B cells and macrophages.

Similar absorption experiments were performed using the spleen cells from *H-2* histocompatible and incompatible strains as well as those from B10 congeneic strains that had been shown to possess no ability to accept the suppressive T-cell factor. As shown in Table VI, the suppressive activity of the BALB/c T-cell factor was completely absorbed with the spleen cells of BALB/c and (BALB/c × CBA)F₁ but not with those from histoincompatible A/J and C3H. It was also shown that the spleen cells of B10.D2 could not absorb the BALB/c T-cell factor even though B10.D2 has the same *H-2* complex as BALB/c. Similarly, the C3H factor can be absorbed with spleen cells of C3H, CBA, (BALB/c × CBA)F₁, and

TABLE IV

*Dominant Expressions of Both the Suppressor and Acceptor Genes on the Cells of (A/J ×
B10.A)F₁*

Donor of T ex-tract	Responding spleen cells	Anti-DNP IgG PFC/culture		Suppression
		Control	with T extract	
				%
(A/J × B10.A)F₁	A/J	2,472 ± 151	618 ± 75	75
(A/J × B10.A)F₁	B10.A	2,994 ± 386	3,467 ± 440	0*
(A/J × B10.A)F₁	(A/J × B10.A)F₁	2,746 ± 554	763 ± 90	72
A/J	(A/J × B10.A)F₁	2,746 ± 554	3,663 ± 238	0*
B10.A	(A/J × B10.A)F₁	2,746 ± 554	729 ± 102	73

* Enhancement.

A/J, but not BALB/c and B10.BR. The results clearly indicate that the T-cell factor can be absorbed with splenic T cells having identical K and I regions to the donor of the T-cell factor, and that the nonacceptor strains of B10 congeneic lines, in fact, do not express the acceptor site for the histocompatible T-cell factors.

Discussion

The results presented in this paper indicate that there exist at least two distinct types of molecules, both determined by genes in the H-2 complex, which play a consequential role in the T-cell-mediated suppression of the antibody response. These are the antigen-specific T-cell factor which has been described previously (1, 2), and the acceptor molecule for the T-cell factor which is expressed on the surface of a certain T-cell subpopulation. This conclusion is derived from a series of experiments all of which point to the dual gene regulation in the T-cell-mediated suppression: First, it has clearly been demonstrated that identities of genes in the H-2 complex between the donor of the T-cell factor and the responding target cells are definitely required for the elicitation of effective suppression. In general, identities among genes in the left side half (K end) of the H-2 complex are found to be both necessary and sufficient. This suggests that the gene(s) coding for the acceptor site is also located in the left side half of the H-2 complex, as is the gene for the T-cell factor, and that the complementation of these two gene products is required for the induction of suppression.

Secondly, in the F₁ hybrid of different H-2 haplotype strains, the molecules having the suppressive activity for both parental strain responses are codominantly expressed on F₁ suppressor T cells. Similarly, the acceptor sites for both parental suppressive T-cell factors are expressed on F₁ target cells. Thus the parental T-cell factors can suppress the response of F₁, and the F₁ T-cell factors can suppress the responses of both parental strains.

The presence of such acceptor sites on T cells but not B cells and macrophages was clearly demonstrated by successful removal of the suppressive activity by incubation of the suppressive T-cell extract with thymocytes and spleen cells, but not with bone marrow cells, peritoneal macrophages, and anti-θ-treated spleen cells. Furthermore, H-2 histoincompatible spleen cells always failed to

TABLE V

Presence of the Acceptor Site for the Suppressive T-Cell Factor on T Cells but not Other Cell Types

Strain tested	T extract absorbed with*	Number of cells	Anti-DNP IgG PFC/culture
C3H	Control	—	1,618 ± 155
	Unabsorbed	—	429 ± 117
	Thymocytes	1 × 10⁸	1,166 ± 218
		2 × 10⁸	1,876 ± 377
	Spleen cells	1 × 10⁸	1,745 ± 113
	Spleen cells treated with anti-θ + C‡	1 × 10⁸	553 ± 131
	Bone marrow cells	1 × 10⁸	496 ± 117
	Peritoneal macrophages	1 × 10⁸	530 ± 100
BALB/c	Control	—	2,895 ± 91
	Unabsorbed	—	420 ± 67
	Thymocytes	1 × 10⁸	1,989 ± 202
		2 × 10⁸	2,771 ± 259
	Spleen cells	1 × 10⁸	2,678 ± 507
	Spleen cells treated with anti-θ + C	1 × 10⁸	587 ± 90
	Bone marrow cells	1 × 10⁸	507 ± 103
	Peritoneal macrophages	1 × 10⁸	567 ± 97

* Absorbed with syngeneic cells.
‡ C, guinea pig complement.

absorb the suppressive activity. The results indicate that the acceptor site is expressed only on the surface of T cells, and this expression is also determined by genes in the *H-2* complex. These findings are consistent with the two-gene model originally presented by Munro and Taussig (6) and Taussig et al. (7) in cooperative interactions between T and B cells in the induction of antibody response, although the acceptor site for the cooperative T-cell signal is expressed only on B cells.

The evidence that the functionally different molecules, i.e. suppressor and acceptor molecules, are actually two distinct gene products was further demonstrated by the occurrence of two types of defect in gene expressions. One example is the A/J strain which could not produce the suppressor molecule, while being able to accept the T-cell factor produced by other *H-2ᵃ* and *H-2ᵏ*

TABLE VI
*Absorption of the Suppressive T-Cell Factor by Histocompatible Spleen Cells,
but not by Those from Nonacceptor Strains of B10 Background*

Donor of T extract	Absorption with spleen cells	Identities of H-2 subregions	Anti-DNP IgG PFC/culture
BALB/c	Control	–	$2,895 \pm 91$
	Unabsorbed	–	420 ± 67
	BALB/c	K, I, S, D	$2,678 \pm 507$
	(BALB/c × CBA)F₁	K, I, S, D	$2,505 \pm 332$
	A/J	S, D	479 ± 60
	C3H	None	572 ± 195
	B10.D2	K, I, S, D	493 ± 73
C3H	Control	–	$1,618 \pm 155$
	Unabsorbed	–	429 ± 117
	C3H	K, I, S, D	$1,745 \pm 113$
	CBA	K, I, S, D	$1,627 \pm 244$
	(BALB/c × CBA)F₁	K, I, S, D	$1,529 \pm 131$
	A/J	K, I	$1,597 \pm 323$
	BALB/c	None	463 ± 103
	B10.BR	K, I, S, D	501 ± 22

strains. Thus, the A/J strain lacks the expression of the suppressor gene, but the expression of the acceptor gene is intact. On the contrary, several congeneic lines of B10 background could produce the suppressive T-cell factor which was effective in suppressing the response of H-2 histocompatible strains of non-B10 background. However, these B10 congeneic mice could not accept the suppressive T-cell factor produced by H-2 histocompatible partners of non-B10 background. The lack of expression of the acceptor site on the spleen cells of B10 congeneic mice was further supported by the consistent failure of absorption of the histocompatible T-cell factor by their spleen cells. Since A/J and B10.A share the identical H-2 haplotype, the observed difference indicates that the expression of suppressor and acceptor genes may be regulated by separate regulatory genes not linked to the H-2 complex.

One further interesting observation in this respect is that the T-cell extract from (BALB/c × A/J)F₁ mice could suppress both syngeneic F₁ and BALB/c responses but not A/J, C3H, and CBA responses. This indicates that although the ability to produce the suppressive T-cell factor is a dominant trait, the produced factor is reactive only with the acceptor of H-2^d. Therefore, the inability of A/J mice to produce the H-2^k reactive T-cell factor is simultaneously inherited by the F₁ mice. However, if A/J (nonproducer) and B10.A (nonacceptor) were crossed to produce their F₁ hybrid, the F₁ mice could produce the factor which can suppress both F₁ and A/J. The results clearly indicate that both expressions of the suppressor and acceptor are dominant traits.

Although the regulatory mechanisms involved in the expression of either molecule are not known, the incidence of such exceptional strains indicates that the suppressor and its acceptor are distinct molecules both coded for by genes in the H-2 complex, and that the suppressor phenomenon is controlled by at least two genes in the H-2 complex. It should be concluded that the complementation of these two gene products is definitely required for the expression of T-cell-

mediated suppression. Defects in either type of gene would cause a defective homeostatic regulation of the antibody response.

In fact, we are aware of the interesting findings reported by Cerottini and Unanue (8), who showed that A/J strain is an extremely high responder to KLH, while CBA is a low responder, and that this responsiveness to KLH is not linked to the *H-2* complex. In view of the present observation, such a high responsiveness observed in A/J mice might be due to the lack of expression of the KLH-specific suppressor gene, although the response itself is not under H-linked *Ir* gene control. It seems possible that the strain differences in the responsiveness against various complex protein antigens may be at least in part explained by the suppressor and/or acceptor gene expression, rather than by *Ir* gene control.

There are several important problems to be solved in the future. Although the T-cell-mediated suppression may be explained by the two-gene hypothesis, the expression of both genes is clearly regulated by other genes not linked to the *H-2* complex. For example, since all the congeneic lines of B10 background so far tested do not express the acceptor site, the expression is apparently regulated by genes on background chromosomes. As C57BL/6J can accept the *H-2^b* factor, the ability to express the acceptor gene is determined by the portion not shared by B10 and C57BL/6J, which is very little indeed. Secondary, although it is now clear that the cells expressing the acceptor site are T cell, we do not know whether they are helper T cells or other cell types. Our more recent observation indicated that the cells capable of absorbing the suppressive T-cell factor are nylon wool-adherent θ-bearing cells. Since most of the helper activity is present in the nonadherent cell fraction, the possibility should be considered that the factor may act on a distinct subpopulation of T cell from helper T cell, and that such cells armed by the factor may in fact be active in the actual suppression of the antibody response. It has recently been proposed by Eardley and Gershon (9) that the suppressor T cell, when transferred into the host, may induce a new population of suppressor cells of the host origin that actually participates in the suppression of antibody response. Thirdly, we are still not able to determine the subregions which code for both suppressor and acceptor molecules. A succeeding paper will describe our latest results which indicate the unambiguous region assignment of the suppressor gene among *I* subregions.[2]

Finally, the present results are in keeping with the recent discoveries of the complementation of two *Ir* genes in the initiation of antibody responses (6, 7, 10–12). These include complementations of genes at the level of T cells and at both T- and B-cell levels. Munro and Taussig (6) suggested that one *Ir* gene codes for a T-cell factor and the other codes for the acceptor on the B cell. More recent studies by Dorf et al. (11) and Dorf and Benacerraf (12) suggest that a complementation of *Ir* gene would occur at the level of T cell. Our results, although the effect was opposite to that observed by these investigators, indicate that the complementation of the suppressor and acceptor genes may take place strictly at the T-cell level, and that the target of the suppressive T-cell factor is the T cell. Another difference from the cooperative cell interaction via *I* region gene

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[2] Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. IV. Special subregion assignment of the gene(s) in the *H-2* histocompatibility complex which codes for the suppressive T-cell factor. Manuscript submitted for publication.

products lies in that there is a strict histocompatibility requirement for the suppressive cell interaction, a fact which was not observed in the study of the cooperative T-cell factor (7). The present results being corroborated by the findings of others would add a strong evidence that several cooperative and suppressive cell interactions are mediated by I region gene products forming the network of the immune system.

Summary

The antigen-specific suppressive T-cell factor of mice, which had previously been shown to be an I region gene product, could effectively suppress the in vitro secondary antibody response of spleen cells from syngeneic or H-2 compatible mouse strains but not that of H-2 incompatible strains. The identities among genes in the left side half (K, I-A, and I-B) of the H-2 complex between the donor and recipient strains were found to be both necessary and sufficient for the induction of suppression. This suggests that the acceptor site for the suppressive T-cell factor is also determined by the gene present in the left side half of the H-2 complex. The cell type which expresses the acceptor site was found to be a subset of T cell. In general, the suppressive T-cell factor obtained from F_1 mice could suppress the responses of both parental strains, and the parental factors could suppress the response of F_1 mice. The results indicate that both suppressor and acceptor molecules are codominantly expressed on F_1 T cells.

There were found two types of defects in the expression of suppressor and acceptor molecules among mouse strains: A/J mice could not produce the suppressive T-cell factor despite that they could accept the factor produced by other H-2 compatible mouse strains. In contrast, all the B10 congeneic lines could produce the T-cell factor, but could not accept the factor produced by syngeneic and H-2 compatible non-B10 congeneic lines. The F_1 hybrid of A/J and B10.A could both produce and accept the T-cell factor, and thus the expressions of suppressor and acceptor molecules were found to be dominant traits. These results indicate that the antigen-specific T-cell-mediated suppression is regulated by at least two genes both present in the H-2 complex, and that the complementation of these two genes is required for the induction of suppression.

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