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

IV. Special Subregion Assignment of the Gene(s) That Codes For the Suppressive T-Cell Factor in the *H-2* Histocompatibility Complex*

By TOMIO TADA, MASARU TANIGUCHI, AND CHELLA S. DAVID

(From The Laboratories for Immunology, School of Medicine Chiba University, Chiba, Japan and The Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110)

Preceding papers in this series indicated that the suppression of antibody response by thymus-derived lymphocytes (T cells) involves a complementary interaction of cell surface molecules of different subsets of T cells which are encoded by genes located within the major histocompatibility (*H-2*) complex of mice (1–5). This has been shown by two lines of experiments: (*a*) The molecule which suppresses the antibody response of syngeneic or *H-2* histocompatible mouse strains can be absorbed with alloantisera raised against the products of genes in the *H-2* complex of the same haplotype. (*b*) The above suppressive molecule of T cells can effectively suppress the antibody response of *H-2* histocompatible mouse strains but not that of *H-2* histoincompatible strains. Thus, the identities among genes in the *H-2* complex between the donor of the suppressive molecule and its target cells are definitely required for the induction of an effective suppression. The results led us to postulate that the suppression by T cells is controlled by at least two genes both located within the *H-2* complex, and a complementary interaction of the products of these two genes plays an essential part of T-cell-mediated suppression of the antibody response (3).

Further studies demonstrated that the suppressive T-cell-derived molecule is, in fact, an *I*-region gene product in $H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^s$ mice (4), although the exact location of such genes in the *I* region was not determined. It was also shown that the acceptor site for the suppressive T-cell factor is encoded by genes in the left side half (*K*, *I-A*, and *I-B* subregions) of *H-2* complex (3–5). Both expressions of suppressor and acceptor genes were found to be dominant traits.

It has been well established that *I*-region genes code for surface molecules on lymphoid and other cell types, which are distinct from *H-2* histocompatibility antigens (6–12). They are designated as Ia antigens being detectable by certain alloantisera raised by immunization of mouse strains sharing only *K* and *D* alleles in the *H-2* complex. The Ia molecules have multiple antigenic determi-

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nants primarily defined by such alloantisera (13). Since the suppressor molecule under investigation has turned out to be an *I*-region gene product, it is necessary to investigate the relationship between the known Ia molecules and the suppressor molecule. Such an approach is also essential to determine the position of the gene or genes that code for the suppressor molecule among the *I* subregions. The present investigation was undertaken to pinpoint the suppressor gene in the *I* subregions by (*a*) absorbing the suppressive T-cell factor with various well-defined antisera against known Ia specificities mapping at different *I* subregions and (*b*) induction of suppression with combinations of donor and recipient strains differing at restricted subregions of the *H-2* complex. The results indicate that the gene(s) that codes for the suppressor molecule is located in a specific subregion (*I-J* subregion) of the *H-2* complex, and that the molecule possesses no previously known Ia specificities.

Materials and Methods

Animals. In addition to the mouse strains used in previous experiments (BALB/cAnN, C3H/HeJ, CBA/J, C57BL/6J, and (BALB/c × CBA)F_1), two recombinant strains with B10 background, i.e., B10.A(4R) and B10.A(5R) were used as producers of the suppressor molecule. B10.A(4R) mice were kindly provided by Doctors Ronald Schwartz and William E. Paul of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., and B10.A(5R) mice were the gifts of Dr. K. Moriwaki of the National Institute of Genetics, Mishima, Japan. All other mice were raised in our animal facility.

Alloantisera. Several alloantisera directed at the restricted subregions of the *H-2* complex were produced at the Department of Genetics, Washington University, St. Louis, Mo. The method of production of these antisera has been published elsewhere (6). Some other alloantisera, B10.A(18R) anti-B10.A(5R), (B10.D2 × A.TH)F_1 anti-A.TL, (A × B10.A(15R))F_1 anti-B10, and (B10 × LP.RIII)F_1 anti-B10.A(2R) were kindly supplied by Doctors B. Benacerraf and M. E. Dorf of the Department of Pathology, Harvard Medical School, Boston, Mass. Other two antisera reactive with Ia.8, i.e. (B10.A × A)F_1 anti-B10 and B10.A anti-B10, were kindly provided by Dr. David H. Sachs of the National Cancer Institute, National Institutes of Health, Bethesda, Md. (C3H.H-2^0 × 129)F_1 anti-C3H (serum designation:D-3b), (B10 × LP.RIII)F_1 anti-B10.A(5R) (serum designation: D-13), and (B10 × A)F_1 anti-B10.D2 (serum designation: D-31) were obtained from The Jackson Laboratory through the courtesy of the National Institutes of Health, Bethesda, Md. All antisera were shipped from the United States to Japan in frozen state. The combinations of mouse strains to produce these antisera and the resultant specificities are shown in the results.

Preparation of the Suppressive T-Cell Extracts and Their Assay Systems. The methods used to obtain the suppressive T-cell factors and to test their activity in in vitro cultured spleen cells have been described in detail in previous publications (2, 3). In brief, the antigen-specific T-cell factor was obtained by physical extraction from thymocytes of mice that had been immunized twice with 100 μg of keyhole limpet hemocyanin (KLH,[1] Calbiochem, San Diego, Calif.) at a 2-wk interval. The cell-free extract, which will be designated at T (thymocyte)-extract, was added to a culture of primed spleen cells at a dose corresponding to 10^7 original thymocytes at the beginning of the culture. In general, 10^7 spleen cells from mice primed with dinitrophenylated KLH (2,4-dinitrophenyl, DNP-KLH) plus 10^9 *Bordetella pertussis* vaccine 4 wk previously were cultured in the Marbrook culture system for 5 days in the enriched Eagle's minimal essential medium with 0.1 μg/ml of DNP-KLH inside of the inner culture chamber.

To test the absorbing capacity of alloantisera for the suppressive T-cell factor, the T-extract was passed through a column of Sepharose 4B immunoadsorbent coupled with gamma globulin fraction of each alloantiserum at 4°C, and the residual suppressive activity of the effluent was assessed by adding to the cultured spleen cells. The antibody response was measured by enumeration of indirect (IgG) plaque-forming cells (PFC) detected by DNP-coated sheep erythrocytes by Cunningham's method after a 5-day culture.

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[1] *Abbreviations used in this paper:* DNP, 2,4-dinitrophenyl; EA, hen's egg albumin; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; T-extract, thymocyte extract.

Results

Lack of Association of the Suppressive T-Cell Factor and Known Ia Molecules. We have previously shown that the suppressive activity of KLH-primed T-cell extracts was successfully removed by absorption with insoluble immunoadsorbents composed of alloantisera directed to the *I* region of the *H-2* complex in $H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^s$ mice (4). Further attempts were made to learn whether or not the suppressive T-cell factor is, in fact, associated with known Ia molecules. Since $H\text{-}2^d$ mice possess Ia specificities Ia.8, 11, and 15 encoded by genes in the *I-A* subregion, and Ia.6 and 7 by genes in *I-C* subregion, the absorptions were carried out using antisera reactive with these antigens. All absorbed materials corresponding to 10^7 original thymocytes were added to the culture of 10^7 DNP-KLH-primed BALB/c spleen cells together with 0.1 μg/ml of DNP-KLH. DNP-specific indirect (IgG) PFC were enumerated after a 5-day culture.

As has been reported previously, two alloantisera raised against homologous haplotype strain (anti-$H\text{-}2^d$) could completely absorb the suppressive activity of the KLH-primed $H\text{-}2^d$ T-extract (Table I). Both antisera were putatively directed against *I-A* and *I-B* subregions of $H\text{-}2^d$, containing anti-Ia.11 antibodies. Although this result suggests that the suppressive activity is associated with Ia.11 specificity, several alloantisera reactive with Ia.8 did not absorb the suppressive activity. Since it has been shown that Ia.11 and 8 are on the same molecule (13), the results exclude the possibility that the activity is associated with the Ia molecule coded for by genes in *I-A* subregion.

Three alloantisera reactive with Ia.6 and/or 7 which are encoded by genes in the $I\text{-}C^d$ subregion were all ineffective in absorbing the suppressive activity, indicating that the active factor is not an Ia molecule determined by genes in the *I-C* subregion. In addition, anti-I^k antisera containing antibodies against shared specificities of Ia.7 and/or Ia.15 had no absorbing capacity. Thus, the suppressor molecule is clearly not the Ia molecule having Ia.6,7 and 15 determinants.

The results indicate that the $H\text{-}2^d$ suppressive T-cell factor is adsorbable by alloantisera directed against $I\text{-}A^d$ and $I\text{-}B^d$ subregions, but apparently there is no association with known Ia molecules. It was also noted that none of the alloantisera detecting shared Ia specificities in a nonhomologous target strain possessed an absorbing activity.

Possible Presence of the Suppressor Gene to the Left of I-C and to the Right of I-B Subregions in $H\text{-}2^k$ Mice. Similar experiments were performed with $H\text{-}2^k$ (C3H) mice using various antisera reactive with *H-2* gene products. The specificities of these antisera with respect to the *H-2* subregions and known Ia determinants are listed in Table II.

As was expected, an antiserum against *H-2K* histocompatibility antigen failed to absorb the suppressive activity of C3H T-extract. On the other hand, most antisera directed to *I* region of $H\text{-}2^k$ were capable of absorbing the suppressive activity. However, as is evident from the putative Ia specificities of these antisera, there is no meaningful relationship between the absorbing capacity of these antisera and their anti-Ia specificities. For example, an antiserum lacking the antibody activity against Ia.3,7 and 15, e.g. (C3H.H-2^o × 129)F_1 anti-C3H, could absorb the suppressive activity, whereas those lacking anti-Ia.1,2,3, and 15, e.g. (B10.A(4R) × 129)F_1 anti-B10.A(2R), were equally effective in absorbing the activity. An antiserum reactive with only Ia.7, e.g. B10.S(7R) anti-

TABLE I

Absorption of H-2^d (BALB/c) Suppressive T-Cell Factor with Various Anti-Ia Antisera Raised Against Homologous (H-2^d) and Heterologous (H-2^b, H-2^k) Haplotype Strains

BALB/c(*H-2*^{d}) T-extract absorbed with:	Specificity		Anti-DNP IgG PFC/culture	Absorption
	H-2 subregions	Ia*		
Control	–	–	2,409 ± 176	–
Unabsorbed	–	–	194 ± 51	–
(B10 × A)F$_1$ anti-B10.D2	K^d, $I\text{-}A^d$, $I\text{-}B^d$	11,16	2,459 ± 100	+
(B6 × A)F$_1$ anti-B10.D2 absorbed with *H-2*^{d} tumor	$I\text{-}A^d$, $I\text{-}B^d$	11,16	2,141 ± 333	+
B10.A anti-B10	K^b, I^b, S^b, D^b	8,(9),(W20)	113 ± 51	–
(B10.A × A)F$_1$ anti-B10	K^b, I^b, S^b, D^b	8,(9),(W20)	395 ± 49	–
(C3H.Q × HTH)F$_1$ anti-C3H.B10	K^b, I^b, S^b	8,(W20)	447 ± 54	–
(A × B10.A(15R))F$_1$ anti-B10	K^b, I^b, S^b	8,(9),(W20)	458 ± 111	–
B10.A(18R) anti-B10.A(5R)	$I\text{-}C^d$	6,7	189 ± 84	–
(B10.A(4R) × HTI)F$_1$ anti-B10.A	$I\text{-}B^k$, $I\text{-}C^d$	6,7	131 ± 7	–
(B10.A(4R) × 129)F$_1$ anti-B10.A(2R)	$I\text{-}B^k$, $I\text{-}C^d$	6,7	281 ± 52	–
A.TH anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^k$	(1,2,3),7,15	307 ± 76	–
(A.TH × B10.HTT)F$_1$ anti-A.TL	$I\text{-}A^k$ $I\text{-}B^k$	(1,2,3),15	311 ± 50	–

* Numbers in parentheses indicate Ia specificities not possessed by *H-2*^{d} strain.

TABLE II

Lack of Association Between the Absorbing Capacity of Anti-Ia Alloantisera and Their Ia Specificities

C3H(*H-2*^{k}) T-extract absorbed with:	Specificity		Anti-DNP IgG PFC/culture	Absorption
	H-2 subregions	Ia*		
Control	–	–	1,798 ± 43	–
Unabsorbed	–	–	288 ± 90	–
A.QR anti-B10.A	K^k	–	278 ± 111	–
(C3H.H-2^o × 129)F$_1$ anti-C3H	K^k, $I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^k$	1,2	1,484 ± 201	+
A.TH anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^k$	1,2,3,7,15	1,777 ± 339	+
(B10.D2 × A.TH)F$_1$ anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^k$	1,2,3	1,621 ± 259	+
(A.TH × B10.HTT)F$_1$ anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$	1,2,3,15	1,516 ± 329	+
(B10.A(4R) × 129)F$_1$ anti-B10.A(2R)	$I\text{-}B^k$, $I\text{-}C^d$	(6),7	1,479 ± 244	+
B10.S(7R) anti-B10.HTT	$I\text{-}C^k$	7	310 ± 199	–
B10.A(18R) anti-B10.A (5R)	$I\text{-}C^d$	(6),7	1,588 ± 75	+
(B10 × LP.RIII)F$_1$ anti-B10.A(5R)	$I\text{-}C^d$, D^d	(6)	2,026 ± 115	+
(B10.K × A.TL)F$_1$ anti-B10.A	$I\text{-}C^d$	(6)	241 ± 57	–

* Numbers in parentheses indicate Ia specificities not possessed by *H-2*^{k} mice.

B10.HTT, was ineffective in absorbing the suppressive activity, while on the other hand, B10.A(18R) anti-B10.A(5R) having the same anti-Ia.7 specificity was capable of absorbing the activity. Thus the absorbing capacity of these antisera had no correlation with their known anti-Ia specificities.

However, there are two important points to be noted from this experiment. Since an active antiserum (A.TH × B10.HTT)F$_1$ anti-A.TL is only directed against the products of *I-A* and *I-B* subregions of *H-2*^{k}, the participation of *I-C* subregion is excluded. Similarly, an antiserum directed against the homologous *I-C*^{k} subregion is ineffective in absorbing the *H-2*^{k} factor. On the other hand, an antiserum (B10.A(4R) × 129)F$_1$ anti-B10.A(2R) which is directed against the products of *I-B* subregion of *H-2*^{k}, can absorb the suppressive T-cell factor. These results indicate that the gene coding for the suppressive T-cell factor maps between *I-A* and *I-C* subregions.

The most interesting fact is that two out of three alloantisera putatively reactive with *I-C*^{d} subregion product can absorb the suppressive T-cell factor of *H-2*^{k} mice. As was shown in the previous section, these antisera lacked the absorbing capacity of homologous *H-2*^{d} T-cell factor (see Table I). The important point is that two of these anti-*I-C*^{d} sera were raised against B10.A(5R), the strain which is a recombinant of *H-2*^{a} and *H-2*^{b}. This puzzling phenomenon can be explained only if we assume that B10.A(5R) strain possesses genes of *H-2*^{k} origin, between *I-B* and *I-C*, originally possessed by B10.A (*H-2*^{a}) strain. This assumption was, in fact, verified by experiments shown below.

Evidence for Separate Molecules in the Suppressive T-Cell Extract of F_1 Hybrid Mouse Which Suppress the Responses of Parental Strains. In a previous publication (3), we have reported that the suppressive T-cell extract of (BALB/c × CBA)F_1 could suppress the responses of both parental strains and *H-2* compatible partners. To assess whether such a suppressive activity for parental strains is associated with a single or separate molecule, we have absorbed the T-cell extract of the (BALB/c × CBA)F_1 with alloantisera directed to *I*-region gene products of *H-2*^{d} and *H-2*^{k}. After absorption with one of such antisera, the residual suppressive activity was assayed by adding the extract to the cultured spleen cells of primed BALB/c and CBA mice.

As shown in Table III, the absorption of the F_1 extract with anti-*H-2*^{d} (anti-*K*^{d}, *I-A*^{d}, and *I-B*^{d}) completely abrogated the suppressive activity for the *H-2*^{d} response, whereas the activity for the *H-2*^{k} response was unaffected. Antisera reactive with *I-C*^{d} subregion gene product did not absorb the suppressive activity for the BALB/c response. Conversely, the passage of the F_1 T-cell extract through the immunoadsorbents of anti-*I*^{k} columns invariably absorbed the suppressive activity for the CBA response leaving the activity to suppress the BALB/c response intact. Here again, the absorbing capacity for *H-2*^{k}-reactive factor was detected in two interesting antisera, i.e., (B10.A(4R) × 129)F_1 anti-B10.A(2R) and B10.A(18R) anti-B10.A(5R). The former is directed against the products of *I-B*^{k} and *I-C*^{d} subregions, and the latter is only reactive with those of *I-C*^{d}. The results are quite consistent with the findings presented in the previous section in that the region that determines the suppressive T-cell factor maps between *I-B* and *I-C* subregion, and that B10.A(5R) mice may contain genes derived from *H-2*^{k} haplotype in this special subregion. The following two experiments will support such an unambiguous subregion assignment of the suppressor gene.

B10.A(4R) Factor Can Suppress the Response of H-2^b *Mice but Not of H-2*^{k} *Mice.* Since it has been firmly established that the suppressive T-cell factor derived from one strain of mice can only suppress the responses of *H-2* histocompatible strains, we have utilized recombinant strains as the sources of the suppressive T-cell factors and tested it in the responses of other strains sharing only restricted *H-2* subregions with the donor strains. B10.A(4R) mice have *H-2*^{k} alleles in *K* and *I-A* subregions and *H-2*^{b} alleles in *I-B*, *I-C*, *S*, *G*, and *D* subregions. Therefore, we have tested the 4R factor in the responses of C3H (*H-2*^{k}) and C57BL/6 (*H-2*^{b}) spleen cells in order to learn the requirement for identities of genes among these subregions to induce a suppression. This would, in turn, allow the implication as to what subregion is responsible for the determination of the suppressive T-cell molecule.

TABLE III

Absorption of the Suppressive T-Cell Factor of (BALB/c × CBA)F_1 with Various Alloantisera Against the I-Region Gene Products of Parental Haplotypes

(BALB/c × CBA)F_1 ($H\text{-}2^{d/k}$) T-extract absorbed with:	Specificity		Anti-DNP IgG PFC/culture	
	H-2 subregions	Ia	BALB/c	CBA
Control	–	–	3,044 ± 273	2,762 ± 124
Unabsorbed	–	–	702 ± 112	773 ± 217
(B10 × A)F_1 anti-B10.D2	K^d, $I\text{-}A^d$, $I\text{-}B^d$	11,16	3,006 ± 272	806 ± 158
(B10.D2 × A.TH)F_1 anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^k$	1,2,3	824 ± 164	2,943 ± 232
(B10 × LP.RIII)F_1 anti-B10.A(2R)	K^k, $I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}C^d$	2,6	902 ± 103	3,410 ± 240
(A.TH × B10.HTT)F_1 anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$	1,2,3,15	907 ± 73	3,353 ± 338
(B10.A(4R) × 129)F_1 anti-B10.A(2R)	$I\text{-}B^k$, $I\text{-}C^d$	6,7	807 ± 79	3,747 ± 198
B10.A(18R) anti-B10.A(5R)	$I\text{-}C^d$	6,7	936 ± 81	2,872 ± 252

Thus, the T-extract derived from B10.A(4R) was added to the culture of C3H and C57BL/6 spleen cells, and the effect was assessed by comparing the numbers of indirect PFC between the control cultures and those with the 4R T-cell extracts. The upper part of Table IV shows the results of this experiment. It was clearly seen that the 4R factor could suppress the response of $H\text{-}2^b$ mice (C57BL/6), whereas the response of $H\text{-}2^k$ (C3H) mice was not suppressed but rather definitely enhanced. Since B10.A(4R) shares the same *I-B* and *I-C* subregions with C57BL/6, the results indicate that the suppressive T-cell factor for $H\text{-}2^b$ mice is determined by *I-B* and/or *I-C* subregions, and that the existing $I\text{-}A^k$ subregion does not contain gene(s) that codes for the suppressive T-cell factor for $H\text{-}2^k$ mice.

Production of the $H\text{-}2^k$ Reactive Suppressive T-Cell Factor by B10.A(5R) Mice. It has been shown that two alloantisera directed to $I\text{-}C^d$ subregion, which were raised against B10.A(5R) mice, could absorb the suppressive T-cell factor of $H\text{-}2^k$ but not $H\text{-}2^d$ mice (see Table I–III). This apparent discrepancy led us to suspect that B10.A(5R) mice may contain a segment originated from $H\text{-}2^k$ haplotype ($I\text{-}J^k$, see below) between $I\text{-}B^b$ and $I\text{-}C^d$ subregions of their *H-2* complex. If it is the case, this strain should aid us in mapping the suppressor gene exactly. Therefore, we have taken the T-extract of KLH-primed B10.A(5R) mice and tested its actiivty in $H\text{-}2^b$ (C57BL/6), $H\text{-}2^d$ (BALB/c), and $H\text{-}2^k$ (C3H) responses.

The results, as shown in the lower part of Table IV, clearly indicate that the B10.A(5R) factor exerted a definite suppressive activity only in $H\text{-}2^k$ response, but not in $H\text{-}2^b$ and $H\text{-}2^d$ responses. Furthermore, the B10.A(5R) factor always enhanced the response of C57BL/6 which shares *I-A* and *I-B* subregions with the donor B10.A(5R). No significant effect was observed in the response of BALB/c mice which shares only *I-C* subregion with B10.A(5R). The results now compel us to conclude that the suppressor gene in B10.A(5R) is located between $I\text{-}B^b$ and $I\text{-}C^d$ subregions (i.e., $I\text{-}J^k$), which is originated from $H\text{-}2^k$ haplotype via $H\text{-}2^a$.

The results presented in Table IV also suggest that *I-A* subregion codes for a distinct molecule which enhances the antibody response of histocompatible partner strains, since the combinations of donor and recipient strains having the same *I-A* subregion but differing in *I-B* and/or *'I-J'* subregions always resulted in a definite enhancement of the antibody response. Identities of *I-C* subregion showed no significant influence on the antibody response.

TABLE IV

Suppressive Activity of the T-Cell Factors from B10.A(4R) and B10.A(5R) in H-2^b, H-2^k, and H-2^d Responses

Donor strain (*H-2* subregions)*	Recipient strain (*H-2* subregions)*	Identities of *H-2* subregions	Anti-DNP IgG PFC/culture		Suppression
			Control	With T-extract	
					%
B10.A(4R) (*k k b b b b b*)	CBA (*k k k k k k k*)	*K,I-A*	2,742 ± 350	3,945 ± 352	0‡
	C57BL/6 (*b b b b b b b*)	*I-B,I-C,S,G,D*	1,838 ± 52	991 ± 46	46
B10.A(5R) (*b b b d d d d*)	C57BL/6 (*b b b b b b b*)	*K,I-A,I-B*	2,369 ± 93	4,307 ± 430	0‡
	CBA (*k k k k k k k*)	None (*I-J* ?)	2,656 ± 109	489 ± 60	82
	BALB/c (*d d d d d d d*)	*I-C,S,G,D*	2,077 ± 221	2,124 ± 244	0

* *H-2* subregions (*K, I-A, I-B, I-C, S, G, D*).
‡ Enhancement.

Possible Existence of a New Sublocus (I-J) Which is Specialized in Coding for the Suppressive T-Cell Factor. Since above studies indicated that the gene that codes for the suppressive T-cell factor maps between *I-B* and *I-C* subregions, the existence of a new subregion *I-J*, which is responsible for the expression of the suppressor molecule, has been suspected. Thus various alloantisera were raised against the *H-2* complex especially to detect the possible *I-J* subregion gene products (Table V). The *H-2^k* (C3H) T-extract was absorbed with these alloantisera, and the residual suppressive activity was likewise tested in the response of syngeneic (C3H) spleen cells. The results were analyzed in relation to the *I-J* specificity of these alloantisera.

The results presented in Table V are, in fact, most reasonably explained by presence of such a new locus between *I-B* and *I-C* subregions, which is specialized in coding for the suppressive T-cell factor. For example, B10.S(7R) anti-B10.S(9R) could absorb the *H-2^k* suppressive T-cell factor, which indicated that B10.S(9R) has '*I-J^k*' subregion between *I-B^s* and *I-C^d*. Conversely, (A.TH × B10.S(9R))F$_1$ anti-A.TL, which theoretically lacks *I-J^k* specificity, could not absorb the *H-2^k* suppressive T-cell factor. However, the other B10.S(7R) antiserum against B10.HTT was unable to absorb the *H-2^k* reactive factor, although B10.S(9R) and B10.HTT have the same *I-A^s* and *I-B^s* subregions. This indicates that unlike B10.S(9R), B10.HTT does not possess I-J^k subregion between *I-B^s* and *I-C^k*.[2]

More striking is the fact that an antiserum B10.A(3R) anti-B10.A(5R) could effectively absorb the *H-2^k* suppressive T-cell factor. Since B10.A(3R) and B10.A(5R) mice are presumed to be identical, it is conceivable that the only difference in the *H-2* complex between these strains is *I-J* subregion.[2] Furthermore, this antiserum did not show any cytotoxic activity for *H-2^k* spleen cells,

[2] The differences between the crossover positions in B10.S(9R) and B10.A(5R) compared to B10.HTT and B10.A(3R), which defined the *I-J* region, were pointed out by Herzenberg and her associates (personal communication) while our work was in progress (see accompanying publication, reference 17).

TABLE V

Possible Presence of a New Subregion (I-J) Which Codes for the Suppressive T-Cell Factor Between I-B and I-C Subregions

T-extract absorbed with*:	Specificities of *H-2* subregions	Anti-DNP IgG PFC/culture	Absorption
Control	–	1,974 ± 87	–
Unabsorbed	–	250 ± 82	–
(A.TH × B10.HTT)F_1 anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, ($I\text{-}J^k$)	1,999 ± 134	+
(A.BY × B10.HTT)F_1 anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$, ($I\text{-}J^k$)	1,975 ± 224	+
(B10.A(4R) × C3H.OH)F_1 anti-B10.K	$I\text{-}B^k$, ($I\text{-}J^k$),$I\text{-}C^k$	2,070 ± 156	+
B10.S(7R) anti-B10.HTT	$I\text{-}C^k$	238 ± 52	–
B10.S(7R) anti-B10.S(9R)	($I\text{-}J^k$),$I\text{-}C^d$	1,932 ± 268	+
(A.TH × B10.S(9R))F_1 anti-A.TL	$I\text{-}A^k$, $I\text{-}B^k$ $I\text{-}C^k$	329 ± 59	–
B10.A(3R) anti-B10.A(5R)	($I\text{-}J^k$)	2,017 ± 192	+

* The $H\text{-}2^k$ (C3H) T-extract was absorbed with alloantisera and the residual suppressive activity was assayed in the response of C3H spleen cells.

which again indicates that the suppressor molecule is in fact distinct from known Ia molecules expressed on B cells.

Discussion

The results presented in this paper clearly indicate that the KLH-specific suppressive T-cell factor is an *I*-region gene product not associated with previously known Ia molecules. This conclusion is derived from the fact that the absorbing capacity of various alloantisera for the $H\text{-}2^d$ and $H\text{-}2^k$ suppressive T-cell factors did not correlate with their known Ia specificities. Furthermore, compelling evidence indicates that the gene coding for the KLH-specific suppressive T-cell molecule maps to the right of *I-B* and to the left of *I-C* subregions, namely in *I-J* subregion. The term *I-J* (suggested by Doctors D. C. Shreffler and H. O. McDevitt) is tentatively assigned to designate the locus coding for the suppressive T-cell factor mapping the gene in a segment of the *H-2* complex existing between *I-B* and *I-C* subregions. Although it has been demonstrated that a new subregion *'I-E'* is present between *I-B* and *I-C* on the basis of serologically detectable Ia-like molecule on spleen cells being coded for by genes in this subregion (David et al., cited in 14), there are considerable differences between the presently described T-cell factor and the Ia-like molecule detected by cytotoxic assays (see below).

The above conclusion has been verified by cumulative evidence as listed below: (*a*) (B10.A(4R) × 129)F_1 anti-B10.A(2R) and (B10.A(4R) × C3H.OH)F_1 anti-B10.K, both of which are directed against the products determined by $I\text{-}B^k$ and $I\text{-}C^d$, invariably absorbed the $H\text{-}2^k$ suppressive T-cell factor, while B10.S(7R) anti-B10.HTT, which is reactive with $I\text{-}C^k$ gene products, did not. This indicates that the *I-A* and *I-C* subregions are not involved in the determination of the suppressive T-cell factor. (*b*) B10.A(18R) anti-B10.A(5R), (B10 × LP.RIII)F_1 anti-B10.A(5R), and B10.S(7R) anti-B10.S(9R), all of which are putatively reactive with $I\text{-}C^d$ gene products, could absorb the $H\text{-}2^k$ factor but not $H\text{-}2^d$ factor. This apparent discrepancy can be resolved by assuming the presence of antibody reactive with $I\text{-}J^k$ subregion gene products in these alloantisera. It was shown that another anti-$I\text{-}C^d$ antiserum (B10.K × A.TL)F_1 anti-B10.A, which

putatively lacks the reactivity with *I-J*^{k} subregion gene product, does not absorb the *H-2*^{k} suppressive T-cell factor. (*c*) The suppressive T-cell extract from B10.A(4R) can suppress the response of *H-2*^{b} but not *H-2*^{k} mice, which also excludes the possibility that *I-A* subregion is responsible for coding for the suppressive T-cell factor. (*d*) In support of the above postulate, B10.A(5R) factor can suppress the response of only *H-2*^{k} mice but not those of both *H-2*^{b} and *H-2*^{d} mice. On the basis of our previous findings that identities among genes in the *H-2* complex are prerequisites for the effective suppression, the results strongly suggest that a chromosomal segment originally derived from *H-2*^{k} haplotype is present to the right of *I-B*^{b} and to the left of *I-C*^{d} subregions in B10.A(5R). (*e*) An antiserum B10.A(3R) anti-B10.A(5R) could absorb the *H-2*^{k} suppressive T-cell factor. Since B10.A(3R) and B10.S(5R) mice are supposed to be identical in *I-A*, *I-B*, and *I-C* subregions, the only difference can be found in *I-J* subregion. This apparently monospecific anti-*I-J* antiserum was unable to kill the splenic B cell, but could effectively remove the suppressive T-cell factor. All these results indicate that the gene that codes for the suppressive T-cell factor maps in the new subregion *I-J* which locates to the right of *I-B* and to the left of *I-C*.

By the close analysis of the data presented in this paper, we are able to type the *I-J* subregion in certain recombinant strains. It is evident that B10.A has *I-J*^{k} subregion, because the B10.A factor could suppress the response of *H-2*^{k} mice but not those of other haplotypes. Since B10.A(5R) could suppress the *H-2*^{k} response, this strain should possess *I-J*^{k}. On the other hand, since B10.A(3R) anti-B10.A(5R) could absorb the *H-2*^{k} suppressive T-cell factor, B10.A(3R) can not possess *I-J*^{k}. Therefore, it is conceivable that the crossover between *H-2*^{a} and *H-2*^{b} has occurred to the left of *I-J* subregion in B10.A(5R), and thus the genes controlling *H-2*^{k} antibody response is preserved in B10.A(5R) mice. On the contrary, in B10.A(3R) the crossover must have occurred to the right of *I-J* subregion, and thus B10.A(3R) should presumably contain *I-J*^{b} locus. The same explanation is also applicable to the apparent difference between B10.S(9R) and B10.HTT; the former has *I-J*^{k} and the latter should have *I-J*^{s} (see Table V).

However, these functional studies do not rule out the possibility that the *I-J* subregion is identical to or a part of *I-E* subregion defined by cytotoxic assays. The presence of *I-E* subregion has been originally proposed by David et al. (cited in Sachs et al., 14) based on the difference in the absorbing capacity of certain anti-Ia antisera between B10.A(5R) and B10.A(3R), both of which are supposed to have the same haplotype. Further evidence for a subregion between *I-B* and *I-C* was obtained when antiserum (C3H.Q × B10.D2)F_1 anti-A.QR reacted with B10.A(5R) (15). Since antibodies reactive with *H-2*^{b} and *H-2*^{d} haplotypes are negative with this antiserum the reaction of B10.A(5R) should be due to a region derived from *H-2*^{k}. Absorption of this antiserum with B10.A(4R) leaves residual activity for B10.A(2R), B10.A(3R), B10.A(5R), B10.HTT, and B10.S(9R), suggesting the presence of *I-E*^{k} region in these strains. The strain distribution of *I-E*^{k} does not correlate with that of *I-J*^{k} and strongly supports the postulate that there are two distinct subregions, i.e., *I-J* and *I-E*, between *I-B* and *I-C* (David, unpublished data).

The presence of such an Ia-like molecule coded for by genes between *I-B* and *I-C* has also been recently suggested by Sachs (16). He showed that B10.A(4R) anti-B10.A(2R) antiserum, after complete absorption with B10.D2, could still

react with B10.A and B10.A(5R) spleen cells. This reactivity was completely abrogated by absorption with B10.BR. Thus, this antiserum contains antibody reactive to the Ia-like molecule determined by genes mapped to the right of *I-B* and to the left of *I-C*, being most likely to be in *I-E* subregion. Since the *I-E* subregion was defined by the serologically detectable Ia determinants which are expressed mostly on B cells, it does not seem to be appropriate to designate the locus that codes for the functional molecule of T cells as *I-E*. Also, it was shown that certain alloantisera lacking cytotoxic activity (e.g., B10.A(3R) anti-B10.A(5R)) could effectively absorb the suppressor molecule.

More recently, Murphy et al. (17) obtained strong evidence that the region that determines an Ia-like molecule on the allotype suppressor T cell also maps between *I-B* and *I-C* subregions, i.e., *I-J* subregion. The molecule on allotype suppressor T cell is different from conventional Ia antigens detected by serological cytotoxic assays for splenic B cells. The absorption of alloantisera with B cells completely removed the cytotoxic activity for splenic B cell leaving the inhibitory effect on the allotype suppressor T cell intact (Herzenberg, personal communication, 17). Therefore, it is most probable that there are two distinct loci between *I-B* and *I-C*, one coding for the suppressor T-cell molecule (*I-J*) and the other for B-cell Ia antigen (*I-E*). Further confirmation should await future comparative studies. From the above findings, it can be concluded that the suppressor gene for KLH-specific T-cell factor maps in this special subregion between *I-B* and *I-C*. It is of interest that both antigen-specific and allotype-specific suppressor T cells carry probably the same *I*-region-determined molecule (17). This raises an important issue that the T-cell-mediated suppression, in general, may be determined by genes present in the newly described *I-J* subregion. These results now clearly distinguish the suppressor genes from previously known *Ir* genes which are mostly mapped in *I-A* subregions. In this regard, the suppressor molecule is also distinguishable from other antigen-specific and nonspecific augmenting T-cell factors (18–22) which have been found to be the products of *I-A* subregion genes.

It has been reported by Debré et al. (23, 24) that a gene or genes controlling the induction of suppressor T cells specific for a random copolymer of L-glutamic acid and L-tyrosine (GT) is present in the *H-2* complex. The term *Is* gene (specific immunosuppression gene) has been originally proposed by them. In veiw of the fact that *H-2*-linked unresponsiveness of mice to a terpolymer of L-glutamic acid-L-alanine-tyrosine (GAT) is mediated by a T-cell product which has similar physicochemical and immunochemical properties to those of ours (25, 26), it is probable that such *Is* genes may well be structural genes which code for the suppressive T-cell factors. Since there have been noted striking similarities between the antigen-induced suppression and genetically determined suppression, it should be important to determine whether the GAT and GT-specific suppressor T cells have *I-J* subregion gene products as functional molecules. Also, it is to be determined whether or not the soluble T-cell factor which mediates the allotype suppression contains *I-J* product. In any event, the relationships between these *I*-region gene products involved in the suppression of antibody responses seem to be of great importance for understanding the suppressor phenomena reported by several investigators with different experi-

mental systems. It should also be important to determine whether or not all the suppressive T-cell factors induced by different antigens are the products of *I-J* subregion genes. Our preliminary studies using a different protein antigen (hen's egg albumin, EA) showed that the EA-specific T-cell factor is also a product of the *I-J* subregion gene.

Finally, the present experiments using recombinant mice showed that combinations of the donor and recipient sharing the same haplotype in *I-A* subregion, but differing in *I-J* subregion, always resulted in the enhancement of antibody response. In view of the studies by Taussig and his associates (19, 20), it is probable that the *I-A* subregion codes for an enhancing T-cell factor, which is distinct from the suppressive factor of *I-J* subregion gene product. It has been reported that both cooperative and suppressive T-cell factors have similar physicochemical properties and antigen-specificity. Our more recent studies indicated that an antigen-specific enhancing T-cell factor does exist in the KLH-primed T-extract, and that the factor is the product of *I-A* subregion gene (unpublished). Furthermore, Okumura et al. (27) showed that some but not all anti-*I*-region antisera can kill helper T cell. The determinant on helper T cell is distinct from that of *I-J* subregion gene product. If these observations are taken collectively, a crucial question can be raised as to what regulatory mechanisms operate the phenotypic expression of genes in either subregion under different conditions on different subsets of T cells. Although no answers are available at the present moment, the finding would provide a clue to studying the enhancing and suppressive T-cell factors both specific for a same single antigen.

Summary

The locus of the gene that codes for the antigen-specific suppressive T-cell factor was determined to be in a new subregion *'I-J'* which locates between *I-B* and *I-C* subregions in the *H-2* histocompatibility complex. This was shown by two different lines of evidence: (*a*) The absorbing capacity for the suppressive T-cell factor of several alloantisera against restricted *I* subregions did not correlate with their specificity for previously known Ia molecules which are coded for by genes in *I-A* and *I-C* subregions, but was associated with the specificity for the products of genes putatively present between *I-B* and *I-C* subregions. By the occurrence of special recombinant strains, i.e. B10.A(5R), B10.A(3R), B10.S(9R), and B10.HTT, which differ with respect to the *I-J* subregion, we were able to produce alloantisera which distinguish *I-J* subregion gene products. The absorption studies using these special alloantisera directed to *I-J* subregion clearly indicated that the suppressive T-cell factor is a product of *I-J* subregion gene(s), and that the molecule is distinct from known Ia molecules expressed on splenic B cells. (*b*) Taking advantage of the fact that there is a strict histocompatibility requirement for the effective suppression between the donor and recipient strains of the suppressive T-cell factor, we were able to determine the required identities of the genes in the *H-2* complex existing among those present between *I-B* and *I-C*. Again, utilizing the T-cell factors obtained from special recombinant strains, i.e. B10.A(4R) and B10.A(5R), we were able to locate the gene that codes for the suppressive T-cell factor reactive only with relevant haplotype strains between *I-B* and *I-C* subregions. These results are most reasonably explained by

the presence of a new subregion *I-J* which is specialized in coding for the suppressive T-cell factor as a different molecule from previously known Ia molecules.

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