I-J-RESTRICTED INTERACTIONS IN THE GENERATION OF AZOBENZENEARSONATE-SPECIFIC SUPPRESSOR T CELLS*

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Regulation of the immune system has been shown (1) to result from complex interactions among subsets of lymphocytes and other cells. Some of these interactions are defined as genetically restricted when the effective interaction requires a certain identity among the cells involved. The genetic restrictions reflect the specificity of the T lymphocyte's receptors and are of two types: (*a*) As originally proposed by Jerne, idiotype specificities determined by Igh-linked genes direct interactions among lymphocyte clones, including T lymphocytes. (*b*) The other set of restrictions concerns the antigens of the major histocompatibility complex (MHC),[1] H-2 in the mouse. Ly-1$^+$ helper and delayed-type hypersensitivity T cells (DTH) are restricted to react to antigen together with gene products of the I-A or I-A-I-E subregions of the murine H-2 complex, whereas cytolytic T cells are committed to interact with K and D specificities. In this study, we analyzed the genetic restrictions controlling the interactions of suppressor T cell subsets.

Suppressor cell circuits in the regulation of T cell dependent antigen-specific DTH have been well studied in several murine systems (2-7). In the sequence of activation of the three major subsets of suppressor T cells (Ts1, Ts2, Ts3), the interaction between second-order suppressor cells (Ts2) and third-order suppressor cells (Ts3) has been shown to be restricted by genes that map to the XIIth (Igh-1 linked) and XVIIth (H-2 MHC) chromosomes (3, 6) of the mouse. The structural elements for Igh-1-linked restriction appear to be clonally expressed, idiotypic determinants present on the antigen-binding cell surface receptors of first-order suppressor cells (Ts1) and also identifiable on soluble suppressor factor produced by Ts1 (TsF$_1$) (5). The same or similar idiotype determinants are present on the cell surface receptors of the Ts3 cell (7). It has been shown that Ts1 or TsF$_1$ stimulates a population of anti-idiotypic Ts2 cells capable of producing and secreting TsF$_2$. TsF$_2$ binds to and triggers Ts3 cells that express the same kind of idiotypic determinants that are on the TsF$_1$ molecule. This idiotypy, therefore, appears to govern certain aspects of Ts subset interactions. On the other hand, although many suppressor T cells express I-J determinants, and I-J restrictions have been reported, little is known about how such a restriction occurs

(5-7) in suppressor T cell interactions. Although TsF_1 and TsF_2 are known to express H-2-encoded determinants (4-6), it is not clear whether these structures function as restriction elements. It is also uncertain whether I-J products present on the Ts1 or Ts2 cells play any special role in immune regulation.

In this report, we present evidence that (a) the I-J-coded determinants on the immunizing cells restrict the specificity of Ts3 cells induced in the course of immunization with azobenzenearsonate (ABA)-conjugated spleen cells, and (b) there is a requirement for identity between the I-J type of the strains where the Ts2 are generated and the I-J type of cells required for activating the Ts3 cells for suppression to be effective.

The significance of these findings will be discussed in terms of the potential role of the I-J subregion gene product in suppressor cell generation. We suggest that I-J gene products may provide an analogous function to the I-A, I-A-I-E subregion gene product required for antigen presentation in positive immune responses (9-11).

Materials and Methods

Mice. A/J (H-2^a, Igh-1^e), A.BY (H-2^b, Igh-1^e), C57BL/6(H-2^b, Igh-1^b), B10.A(5R) (H-2^{i5}, Igh-1^b), and (A.By × A/J)F$_1$ (H-$2^{a/b}$, Igh-1^e) B10.Br (H-2^k, Igh-1^b) B10.D2 (H-2^d, Igh-1^b) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.A(3R) (H-2^{i3}, Igh-1^b) B10.A(4R) (Igh-1^b), and B10.A(5R) were also obtained from Dr. Martin Dorf, Harvard Medical School, Boston, MA, and were maintained in our animal facility at the Department of Pathology, Harvard Medical School. All strains of mice were used at 8-12 wk of age.

Antigen Presentation and Immunization. Erythrocyte-free spleen cells were conjugated with ABA by incubating with activated ABA diazonium salt solution at a final concentration of 10 mM, as described previously (9). For immunization, 3×10^7 ABA-coupled spleen cells (ABA-SC) were injected subcutaneously into two separate sites on the dorsal flanks of the mice.

Assay for DTH and Cytotoxic T Lymphocytes (CTL). 5 d after immunization, mice were challenged by injecting 30 μl of 10 mM ABA-diazonium salt solution into the left hind footpad. The footpad swelling was measured 24 h later (9). For CTL assay, spleens were aseptically removed from mice 6-7 d after immunization. Spleen cells were restimulated in vitro for 5 d with irradiated ABA-SC. Culture condition was 7×10^6 responder lymphocytes and 6×10^6 stimulator cells in 1 ml RPMI 1640 plus 5% fetal calf serum (FCS), 5×10^{-5} M 2-mercaptoethanol in each well of Costar 24-well tissue culture trays. The cells were kept in a humidified, 5% CO_2 atmosphere. Cytolytic activity against ABA-coupled YAC-1(H-2^a) or EL-4(H-2^b) target cells was measured by 4 h ^{51}Cr release assay (10).

Suppressor Factors. Either TsF_1 extracted from spleen cells of A/J mice that had been injected with 5×10^7 ABA-SC 7 d earlier (4) or monoclonal suppressor factor produced by Ts1 hybridoma of A/J origin, F12 (9), was used to induce TsF_2. A total of 10^8 cell equivalents of conventional first-order T cell suppressor factor (TsF_1) or 1 ml of F12 culture supernatant was injected intravenously into various strains of mice daily for 5 d. 2 d after final injection, a spleen cell suspension at 5×10^8 cells/ml in Hanks' balanced salt solution was prepared and underwent five cycles of freezing and thawing. Supernatant was collected after spinning at 12,000 rpm for 90 min and stored at $-20°C$ up to 2 mo. TsF_2, thus prepared, are designated by the strain of mice that were injected with TsF_1 of A/J origin and when the Ts2 were generated. For example, TsF_2-A.By indicates that the Ts2 cells used to prepare TsF_2 were induced in A.By mice. A total of 6×10^7 cell equivalents of TsF_2 was injected intravenously on days 4 and 5 after immunization. Because TsF_1 requires its administration at the time of immunization to express suppressive activity, this protocol enables us to exclude the carry-over effect of transferred TsF_1.

Results

I Region-disparate ABA-coupled Cells Cannot Prime Mice to Be Suppressed by TsF_2. We have previously shown that TsF_2, obtained from mice given TsF_1, mediates suppression

by triggering an idiotype-bearing antigen-primed T cell present in the immune population. This cell is the Ts3 cell. To determine whether the activation of Ts3 cells, after immunization with ABA-coupled cells is restricted, B10.A, B10.Br, and B10.A(4R) cells were coupled with ABA and used to prime A/J mice. Previous studies (9) had indicated the effectiveness of H-2-congenic hapten-coupled cells in ABA priming; in those studies, I-A region identity was found to be necessary for ABA-specific T cell sensitization. As shown in Table I, immunization with ABA-coupled B10.A or B10.Br causes the activation of T_{DH} cells and Ts3 suppressor cells that could, upon interaction with A/J TsF$_2$, result in significant suppression of ABA-specific T_{DH} activity. B10.A(4R)-coupled cells, although capable of stimulating T_{DH} cells, could not induce Ts3 cells capable of suppressing ABA DTH when A/J TsF$_2$ was simultaneously administered. Because B10.A(4R) differ in the IB-D regions and B10.Br differ in IC-D from A/J, we conclude from this experiment that I region gene products in the I-B, I-J, or IE regions must match between the immunizing inoculum for Ts3 sensitization and the TsF$_2$ for effective suppression.

Selective Suppression by Parental TsF$_2$ in F$_1$ Mice Primed with either Parental Cells Coupled with ABA. We then evaluated the activation and triggering signals in two ways, namely, using matching of the priming cells and the recipient, and second, by evaluating the H-2-restricted interaction of TsF$_2$ with the priming cells in greater detail.

Because Ts3 is activated during the course of immunization, attempts were made to further document the requirement for restricted antigen presentation in the effective generation of Ts3 and to evaluate any special relationship between TsF$_2$ and the immunizing cells. (A.By × A/J)F$_1$ mice were immunized with either ABA-coupled A/J cells or ABA-coupled A.By cells and injected with either TsF$_2$-A/J or TsF$_2$-A.By, and the subsequent ABA-specific DTH and CTL responses were assayed.

As shown in Figs. 1 and 2, suppression was selectively limited to the H-2 type of ligand-coupled cells used for priming of Ts3. Effective suppression was only observed when ABA-coupled cells and TsF$_2$ had the same genetic origin. This suggests that the Ts3 cells are selected by antigen and some H-2 determinants expressed on the antigen-coupled cells to be prepared for triggering by TsF$_2$. Furthermore, when (A.By × A/J)F$_1$ animals were immunized with ABA coupled to (A.By × A/J)F$_1$ cells, the ABA-specific DTH and CTL responses were equally suppressed by either TsF$_2$-

TABLE I

I Region-disparate ABA-coupled Cells Cannot Prime Mice to Be Suppressed by TSF$_2$

Immunization*	H-2 formula K ABJEC SD	Treatment‡	Footpad swelling × 10^{-2} mm
ABA-B10.BR	k kkkkk kk	—	21.25 ± 3.3
ABA-B10.BR	k kkkkk kk	TsF$_2$	5.7 ± 1.6 ($P < 0.01$)
ABA-B10.A	k kkkkd dd	—	26.5 ± 1.4
ABA-B10.A	k kkkkd dd	TsF$_2$	10.5 ± 1.8 ($P < 0.01$)
ABA-B10.A (4R)	k kbbbb bb	—	24.25 ± 2.6
ABA-B10.A (4R)	k kbbbb bb	TsF$_2$	19.25 ± 2.8 (NS)§

* Groups of A/J mice were immunized with 3 × 10^7 ABA-conjugated B10.BR or B10.A or B10.A (4R) spleen cells.

‡ 5 d later, some of these mice were injected with an A/J TsF$_2$ prepared from Ts2 induced by A/J TsF$_1$. (5 × 10^7 cell equivalent) 1 d before challenge.
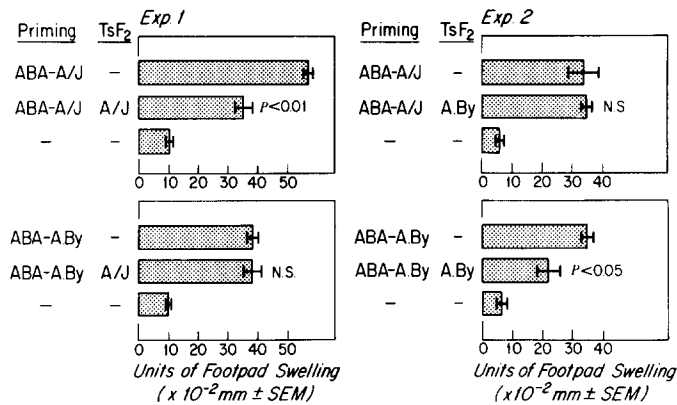
§ Not significant.

FIG. 1. Selective suppression of ABA-specific DTH in (A.By × A/J)F₁ mice by parental TsF₂. (A.By × A/J)F₁ mice were immunized subcutaneously on day 0 with either ABA-SC of A.By or ABA-SC or A/J. A total of 6 × 10⁷ cell equivalents of either TsF₂-A/J (experiment 1) or TsF₂-A.By (experiment 2) was injected intravenously on days 4 and 5. Challenge with 30 μl or 10 mM ABA was performed on day 5, and 24 h footpad swelling was assessed.
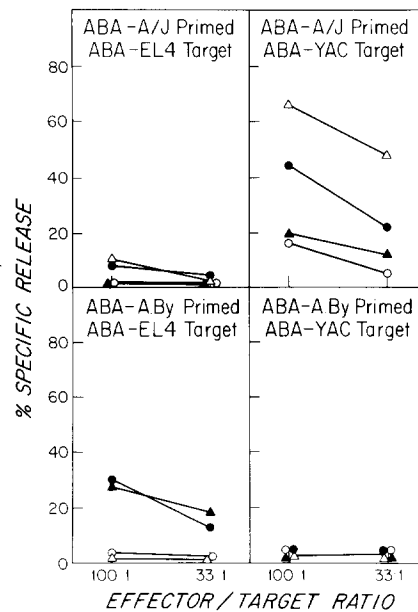

FIG. 2. Selective suppression of ABA-specific CTL generation in (A.By × A/J)F₁ mice by parental TsF₂. (A.By × A/J)F₁ mice were immunized subcutaneously on day 0 with 3 × 10⁷ of either ABA-SC or A/J or ABA-SC or A.By. A total of 6 × 10⁷ cell equivalents of either TsF₂-A/J or TsF₂-A.By was injected intravenously on days 4 and 5, and spleens were removed on day 7. For 5 d, 7 × 10⁶ cells were incubated with 6 × 10⁶ ABA-coupled and irradiated spleen cells of (A.By × A/J)F₁ in 1 ml of medium. Cytotoxicity against ⁵¹Cr labeled-ABA-coupled YAC-1 (H-2ᵃ) cells and EL-4(H-2ᵇ) cells was determined by 4-h release assay. Positive control (●), TsF₂-A/J (▲), and TsF₂-A.By (△). The negative control (○) did not receive in vivo immunization but was stimulated in vitro for 5 d in the same way as the other groups.

A.By and TsF₂-A/J (data not shown).

To evaluate the role of I-J in the TsF₂, we obtained TsF₂ from B10.A(3R) or B10.A(5R) mice. Suppression with TsF₂-B10.A(5R) and TsF₂-B10(3R) was tested by the same protocol. TsF₂-B10.A(5R) suppressed only the response of (A/J × A.By)F₁

TABLE II

I-J Restriction in Selective Suppression by TsF_2 in $(A.By \times A/J)F_1$ Hybrid Mice

TsF_2 induced in*	H-2 formula K ABJEC SD	Percent suppression for ‡	
		ABA-A/J primed	ABA-A.By primed
B10.A (3R)	b bbbkd dd	3.2	50.2
B10.A (5R)	b bbkkd dd	56.7	12.1

$(A.By \times A/J)F_1$ mice were immunized subcutaneously with 3×10^7 ABA-SC of either A/J or A.By on day 0. TsF_2 were injected on days 4 and 5 at a total dose of 6×10^7 equivalents. Challenge with 30 µl of 100 mM ABA was performed on day 5, and 24-h footpad swelling was assessed.

* TsF_2 were induced in B10.A(5R) and B10.A(3R) by 5 daily intravenous injections of F12 supernatant at a total of 1 ml.

‡ Percent suppression is ([positive − TsF_2 group]/[positive − negative]) × 100.

TABLE III

Immunization with I-J-disparate ABA-coupled Cells Prevents Suppression by TsF_2

Immunization*	H-2 formula K ABJEC SD	TsF_2 treatment‡	Footpad swelling $\times 10^{-2}$ mm§
ABA B10.A (5R)	b bbkkd dd	—	26 ± 3
ABA B10.A (5R)	b bbkkd dd	B10.A (5R)	10.6 ± 3.6 $(P < 0.01)$
ABA B10.A (5R)	b bbkkd dd	B10.A (3R)	20 ± 2.3 $(P < 0.25)$
ABA-B10.A (3R)	b bbbkd dd	—	25 ± 2.3
ABA-B10.A (3R)	b bbbkd dd	B10.A (3R)	10 ± 2.6 $(P < 0.018)$
ABA-B10.A (3R)	b bbbkd dd	B10.A (5R)	21 ± 1.3 $(P < 0.36)$
Challenge only		—	7.6 ± 1.3

* $(C57Bl/6 \times A/J)F_1$ (bbbbbbbb × kkkkkddd)F_1 mice were immunized subcutaneously with 3×10^7 ABA-SC of either B10.A (5R) or B10.A (3R) on day 0.

‡ TsF_2 prepared in B10.A (3R) or B10.A (5R) mice using F12 (see Table II) were injected on days 4 and 5 at a total dose of 6×10^7 equivalents.

§ Challenge was performed using 30 µl of 100 mM ABA on day 5, and 24 h footpad swelling was assessed.

mice primed with ABA-coupled A/J cells, and TsF_2-B10.A(3R) suppressed only when F_1 mice were immunized with ABA-coupled A.By cells (Table II). Thus, I-J identity between the TsF_2 and the immunizing cells is required for suppression.

The results of the CTL assay (Fig. 2) also provide important information with respect to the effect of in vivo antigen priming. The priming with ABA-coupled cells was absolutely specific. For example, spleen cells of $(A/J \times A.By)F_1$ mice primed with ABA-coupled A/J cells only killed ABA-coupled YAC-1($H-2^a$) target cells but not EL-4($H-2^b$) target cells. Furthermore, we also showed that ABA-specific proliferative responses in the F_1 hybrid could be selectively activated in a haplotype-specific manner using the same protocol as described here (data not shown). These data strongly suggest that no representation of antigens occurred for activation of either CTL effector cells, T proliferative cells, and Ts3 cells. Collectively, these results indicate that there is a necessity for I-J matching between the TsF_2 and the ABA-coupled cells used to induced Ts3 for suppression to occur.

Mapping of the Inductive and Triggering Signals for Ts3 Functions. To establish the I region restrictions for the activation of Ts3 cells, the following experiments were done. $(C57BL/6 \times A/J)F_1$ mice were primed with ABA-coupled B10.A(3R) or B10.A(5R)

TABLE IV

Suppression of Cytotoxicity Is I-J Restricted

Immunization*	H-2 formula K ABJEC SD	TsF₂ treatment‡	Percent specific release 11:1 effector-to-target ratio§	Lytic units ‖
ABA-B10.A (3R)	b bbbkd dd	—	18	3.5
ABA-B10.A (5R)	b bbkkd dd	—	18	3.6
ABA-B10.A (3R)	b bbbkd dd	B10.A (3R)	0	0.0
ABA-B10.A (3R)	b bbbkd dd	B10.A (5R)	13	2.5
ABA-B10.A (5R)	b bbkkd dd	B10.A (3R)	15	3.1
ABA-B10.A (5R)	b bbkkd dd	B10.A (5R)	2	0.4
—	—	—	1	0.2

* Immunization as in Table III.
‡ TsF₂ was prepared and used in the same manner as Table III.
§ 7 × 10⁶ B6AF₁ responders were cultured for 5 d with 6 × 10⁶ A.By irradiated stimulators in 2 ml RPMI with 1% FCS. Effectors were harvested and tested for ability to lyse ⁵¹Cr-labeled A.By Con A blast target cells in standard 4 h ⁵¹Cr release assay.
‖ Lytic unit is defined as the number of spleen cells required to give 50% specific release from 10⁴ target cells in 4 h.

cells. This strain combination permits us to evaluate the role of I-J, while it minimizes any significant allogeneic effects and permits the necessary I-A region identity for ABA priming. Groups of these mice received TsF₂ induced in either B10.A(3R) or B10.A(5R) mice. The mice were evaluated for ABA-specific responses on day 5. The data shown in Tables III and IV demonstrate the requirement for I-J matching of the hapten-coupled cells used to prime mice for Ts3 and the TsF₂ necessary to trigger these cells to manifest suppression.

Discussion

The major finding in this report is the genetic restriction of Ts3 activation and function, illustrated by the special relationship between the TsF₂ molecule and the ABA-conjugated cells required to activate Ts3. To activate Ts3 cells that, in the ABA system, appear to be the final effector suppressor cells, two events are required. First, the Ts3 must be induced by appropriately presented ligand at the time of priming, and, second, TsF₂ or a similar signal must trigger the primed cell. The Ts3 cells induced appear to require ligand presented by cells that are I-J identical to the TsF₂ subsequently used for complete suppression to occur. Thus, I-J gene products may be critical for the induction and triggering of Ts3 cells, and this might indicate that I-J gene products play a critical role as a restriction element in the suppressor pathway.

In the ABA system, two clusters of genes are important in the suppression. The Igh-1-linked set of genes encode idiotype-like structures that govern certain levels of interaction between members of this pathway. Ts1 cells induced by ligand in certain strains, e.g., A/J, express idiotype-like structures and elaborate idiotype and I-J-bearing molecules, termed TsF₁. This TsF₁, in the absence of further antigen, can induce anti-idiotypic Ts2 cells in any recipient strain. Ts2 cells or TsF₂ derived from these cells must, however, interact with antigen-primed idiotype-bearing Ts3 cells for

suppression to occur. Furthermore, we have also shown that the anti-idiotypic Ts2 and its idiotype target must be H-2 identical. Specifically, this is illustrated by our observation that, when A/J TsF$_1$ is used to induce Ts2 in A.By (H-2^b, Igh-1^e) or C57Bl/6 (H-2^b, Igh-1^b), the Ts2 can be shown to be active only in A.By (H-2^b, Igh-1^b) and do not function in A/J or C57Bl/6. C57Bl/6, although H-2 identical to A.By, does not express cross-reactive idiotype. In this study, attention to the rules of matching between the TsF$_2$ and Ts3 cells at the H-2 and Igh-1 region has permitted us to identify the role of I-J-coded determinants in these interactions.

To account for the requirement of I-J identity between the TsF$_2$ and the cells used to induce Ts3, we propose that the Ts3 cells, in addition to ligand, recognize an I-J determinant on certain hapten-coupled cells. This I-J structure is apparently the same that is recognized on the anti-idiotypic I-J-bearing TsF$_2$ molecule. Thus, the Ts3 cell behaves as an analogue of the murine Ly-1$^+$ T cell subset described by Schwartz and Paul (11) and others, which recognize I-A, I-A-I-E gene products and antigen on the surface of specialized antigen-presenting cells. If this is the case, I-J gene products are restriction elements for Ts3 activation.

This interpretation is supported by the results from the experiments described herein, in which the (C57Bl/6 × A/J)F$_1$ immunized with either B10.A(3R) or B10.A(5R) cells coupled to antigen failed to be suppressed by the TsF$_2$ induced in the I-J-disparate strain. In other words, Ts3 are activated by certain I-J gene products on the immunizing cells in addition to the haptenic determinant. These determinants must be identical with I region gene products of the host in which Ts2 is induced. However, we have not yet obtained structural evidence that the H-2 product on the immunizing cell is the I-J antigen. These experiments are currently in progress using monoclonal anti-I-J antibodies.

Involvement of molecules encoded by the I-J subregion in immunological suppression have been considered for several years. Indeed, I-J restrictions were initially reported in the elegant studies of Tada and colleagues (12). In addition, most of the Ts and their soluble mediator molecules bear I-J-coded antigens. Certain I-J region-encoded antigens have also been shown to be present on some other nonsuppressor subsets of cells (13–17). Functional associations of I-J subregion and suppression were also recently reported for the phenomena in which allogeneic interactions across I-J subregion differences results in the suppression (18–19). These studies indicated that anti-I-J-I-J interactions could trigger Ts1 or Ts2 cells (20). More recently, I-J-specific (i.e., anti-I-J) allogeneic effect factor has been shown to provide the activation signal for a certain subset of Ts (21). As presented here, the function of TsF$_2$ is also restricted to the I-J subregion. In this regard, similar I region restrictions have also been reported for suppression induced by Ts cells in the nitrophenyl system (22). In other antigen systems, I-C (23) and I-J (24, 25) regions have all been shown to individually determine restrictions.

It is generally considered that antigens must be presented on antigen-presenting (APC) cells to stimulate immune reactivity effectively. APC activity seems to require expression of I region-associated (Ia) antigen and ligand. In some experimental systems, spleen cells chemically coupled with haptenic determinants are also effective as APC. The evidence for the requirement of Ia, or more precisely I-A subregion-encoded antigens in the ABA system for effective presentation has been accumulated; ABA-coupled spleen cells that shared I-A subregion with the responding animal were

effective in the immunization for ABA-specific DTH (9), and the immunization was inhibited by the administration of anti-I-A antibodies into the animal (26). Treatment of APC with monoclonal anti-I-A antibody and complement completely prevents in vitro ABA-specific CTL activity (A. Tominaga and M. I. Greene, manuscript in preparation). Abrogation of immunity by anti-I-A antibodies has also been recently shown in several other antigen systems (28, 29). Moreover, monoclonal anti-I-A antibodies coupled with ABA induced unresponsiveness rather than inducing DTH (27). Thus, activational signals associated with I-A molecules are required for the induction of immunity, and the lack of these signals seems to lead to unresponsiveness (27–29).

Antigen presentation for activation of Ts, on the other hand, has not been previously considered. It has been simply assumed that the antigens that escaped trapping, processing, and presentation by the reticuloendothelial system (RES) directly bound to and activated Ts. This is based on several observations (30) that the form and route of administration of antigen that led to limited handling by the RES, i.e., such as intravenous administration of cell-bound antigens, etc., were effective inducers of tolerance and suppression. Such Ts were, however, always evaluated in animals or systems that had been primed with antigen. Because the Ts3 activation and subsequent interaction with TsF_2 molecules appear to be a major site of I-J restriction, it is not surprising that I-J presentation was not formally identified in these aforementioned studies. Results presented in this report suggest, however, the existence of I-J-related antigen presentation for the activation of at least a certain subset of Ts (Ts3). This presentation appears to be an analogous counterpart of I-A- or I-A-I-E-related antigen presentation necessary for helper or DTH immune responses. However, in some unexplained manner, the contribution of I-J-positive APC may also be relevant for certain positive immune responses (16). Indeed I-J$^+$ APC cells may quantitatively and qualitatively govern many aspects of the immune response.

Summary

The genetic restrictions of the activation of third-order suppressor cells (Ts3) were studied in mice, using two different types of anti-azobenzenearsonate (ABA)-immune responses, namely delayed-type hypersensitivity (DTH) and cytotoxic T lymphocyte (CTL) generation. Ts2 cells were induced in several different strains of mice by injecting monoclonal T hybridoma molecules or first-order suppressor factors (TsF_1) originating in A/J ($H-2^a$, Igh-1^e) mice and then testing the TsF_2 molecules derived from these Ts2 in A/J and A.By ($H-2^b$, Igh-1^e) or (A/J × A.By)F_1 ($H-2^{a/b}$, Igh-1^e) and (C57Bl/6 × A/J)F_1 ($H-2^{b/a}$, Igh-1$^{b/e}$) mice. It was shown that the activity of TsF_2 was restricted to the I-J of the strain in which Ts2 was induced. By genetic analysis, restriction was shown to be due to the requirement of H-2 identity between ABA-coupled cells used for Ts3 activation and the strain of the TsF_2 origin. Moreover, by using H-2-congenic ABA-coupled cells, we were also able to precisely map and demonstrate that ABA-coupled cells I-J identical to TsF_2 induced in various strains were necessary for effective suppression to occur. This selective activation of Ts3 suggested the existence of I-J-related antigen presentation for suppression as the counterpart of I-A or I-A-I-E-restricted antigen presentation for positive immune

responses.

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