ACCEPTOR-SUPPRESSOR T CELL HYBRIDOMA WITH A RECEPTOR RECOGNIZING ANTIGEN-SPECIFIC SUPPRESSOR FACTOR*

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Immune responses are known to be regulated by complicated interactions of T cells with different functional roles. In our previous studies of the cellular mechanism of suppression of antibody response in the keyhole limpet hemocyanin (KLH)[1] system, we have demonstrated that at least three distinct subsets of suppressor T cells are involved in the regulatory T cell interactions (1).[2] These T cell populations involve suppressor T cells that produce antigen-specific suppressor T cell factor (TsF), suppressor T cells bearing a receptor for TsF (acceptor-suppressor T cells), and effector-suppressor T cells. The KLH-specific TsF with antigen-binding moiety and I-J determinants, which is composed of two polypeptide chains, is obtained from $Lyt-2^+$ suppresor T cells. In the initial step of the suppressor pathways, the KLH-specific suppressor T cell factor (KLH-TsF) acts on and activates $Lyt-1^+,2^+$ acceptor-suppressor T cells. In the second step, the activated acceptor-suppressor T cells generate new effector-suppressor T cells that directly suppress the responses mounted by B cells and helper T cells. Therefore, the antigen-specific TsF seems not to be an effector-suppressor molecule but rather appears to work as a signal to activate the family of suppressor T cells.

The existence of acceptor-suppressor T cells was demonstrated by the absorption of the TsF activity with $Lyt-1^+,2^+$ splenic T cells in the absence of relevant antigen and by the loss of the TsF function after treatment of acceptor T cells with anti-Lyt-1 or anti-Lyt-2 antisera and complement.[2] Similar populations of acceptor-suppressor T cell types have been described in several other suppressor cell systems (2-5) specific for 4-hydroxy-3-nitrophenyl acetyl (NP), L-glutamic acid^{50}-L-tyrosine^{50} (GT), and p-azobenzenearsonate (ABA), etc., in which the antigen-binding, idiotype-positive TsF acts on and stimulates the second order of acceptor-suppressor T cells bearing $Lyt-1^+,2^+$ phenotypes and antiidiotype

receptors for the idiotype-positive TsF. The activated second-order acceptor T cells then function to stimulate the Lyt-2$^+$ precursor cells to activate effector-suppressor T cells mediating suppression in the effector phase of the immune response. It is thus suggested that the acceptor-suppressor T cells of the intermediary type play decisive roles in amplifying suppressor signals in regulatory T cell interactions. In this sense, the use of monoclonal TsF and suppressor T cell hybridomas of the acceptor cell type permits us to analyze this amplification mechanism, mediated by TsF-acceptor T cell interaction in the suppression of antibody response, at molecular levels. We describe here the generation and characterization of hybridomas of the acceptor T cell type that bear a receptor for the KLH-TsF composed of two polypeptide chains. The acceptor hybridoma cells fail to express any functional activity by themselves, but start to produce another "KLH-specific" suppressor factor possessing the complementary structure that recognizes the KLH-TsF when the cells are stimulated with monoclonal or conventional KLH-TsF of the two-chain type in the absence of the relevant antigen KLH. The idiotype-antiidiotype network in the KLH-specific suppressor system is discussed.

Materials and Methods

Animals.   C57BL/6 CrSlc, and C3H/HeJ CrSlc mice were purchased from Shizuoka Experimental Animal Laboratory Co., Ltd., Hamamatsu, Japan.

Antigens.   KLH was purchased from Calbiochem-Behring Corp., San Diego, CA. Oval-bumin (OVA), recrystalized five times, was obtained from Sigma Chemical Co., St. Louis, MO. Dinitrophenylated KLH (DNP-KLH) and OVA (DNP-OVA) were prepared by coupling with 2,4-dinitrobenzenesulfonic acid under alkaline conditions. *Bordetella pertussis* vaccine (BPV) was obtained from the Chiba Serum Institute, Chiba, Japan.

Antiserum.   Conventional .alloanti-I-J^b, B10.A(5R) anti-B10.A(3R) was raised in our laboratory by immunization with mitomycin C-treated spleen cells and thymocytes, as described elsewhere (6). Mouse anti-KLH and anti-OVA antisera were produced by immunization of C57BL/6 mice intraperitoneally once or twice with 100 μg KLH or OVA and 1×10^9 BPV.

Preparation of Suppressor Factors.   Monoclonal KLH-TsF was obtained in the cell-free extracts of suppressor T cell hybridoma cells (34S-18, 34S-704, and 1L-1) by freezing and thawing (extracted TsF) or in the ascitic fluid of hybridoma-bearing mice (secreted TsF) as described elsewhere (7). The conventional KLH-TsF or OVA-TsF was prepared by freezing and thawing of the thymocytes from C57BL/6 mice primed twice with 200 μg of KLH or OVA at 2-wk intervals as described (1). KLH-TsF has been found to be composed of two polypeptide chains, one having the KLH-binding moiety and constant region determinant and the other carrying the I-J determinant detected by B10.A(5R) anti-B10.A(3R) as described elsewhere (6–8).

Hybridization and Screening of the Acceptor Hybridoma Clone.   The acceptor hybridoma clone, 34S-281, was established by the fusion of BW5147 and KLH-primed splenic suppressor T cells enriched with KLH-coated petri dishes. This protocol was exactly the same as that to obtain KLH-TsF-producing hybridomas as previously reported (7). After cloning with limiting dilution, the candidates for acceptor hybridoma cells were screened by functional assay to determine whether the cloned hybridoma had the ability to absorb the activity of the monoclonal KLH-TsF. The 34S-281 hybridoma line was cloned by limiting dilution several times. The hybridoma was cultured and maintained in HAT medium (RPMI 1640 containing 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, 1.6 $\times 10^{-5}$ M thymidine) enriched with 10% fetal calf serum. The hybridoma line was unstable. Therefore, the cells were recloned with limiting dilution and were selected by testing the absorbing capacity of KLH-TsF every 1–2 mo. By this procedure, the

hybridoma clone, 34S-281, has been maintained for >5 yr.

Surface Phenotypes of the Cloned Acceptor Hybridoma. The cloned acceptor hybridoma (34S-281) was killed by alloantisera of anti-H-2^b (C3H anti-C3H.SW), anti-H-2^k (C57BL/6 anti-C3H), and anti-I-J^b B10.A(5R) anti-B10.A(3R)). Moreover, the hybridoma was killed by the anti-I-J^b absorbed with spleen cells of B10.A(5R), C3H, or BALB/c, while the anti-I-J^b absorbed with C57BL/6 and B10.A(3R) spleen cells failed to kill the hybridoma, indicating that the clone 34S-281 is a hybridoma of H-2^k and H-2^b mice and expresses I-J^b determinants. Concerning the expression of Thy-1.2 antigens, the cells were susceptible to the treatment with monoclonal or conventional anti-Thy-1.2. However, the hybridoma cells have not shown Thy-1.2 expression since 2 yr after the hybridization.

Absorption of Suppressor Factor with Hybridoma Cells. 1 × 10^7 hybridoma cells or BW5147 thymoma cells were incubated, with 100 μl of the ascitic monoclonal KLH-TsF or the factor derived from activated hybridoma cells, for 1 h on ice with occasional mixing. The process was repeated three times. The supernates were harvested by centrifugation at 400 g for 10 min and tested for suppressor activity in the in vitro anti-DNP IgG secondary antibody response of C57BL/6 spleen cells primed with DNP-KLH.

Cytotoxic Test. The two-step dye exclusion cytotoxic assay was performed as previously described (6).

Activation of Acceptor Hybridoma with KLH-TsF. 1 × 10^7 acceptor hybridoma cells (34S-281) were incubated with monoclonal KLH-TsF (either cell-free extracts equivalent to 2 × 10^6 or 100 μl of ascitic factor) on ice for 1 h in the absence of relevant antigen. The cells were washed three times with RPMI 1640 and then cultured in RPMI 1640 with 10% fetal calf serum. As controls, the hybridoma cells were treated with culture medium under the same conditions as the experimental groups. The cell-free extracts or the culture supernates from the activated or nonactivated acceptor hybridoma cells were prepared at different time intervals after the stimulation with KLH-TsF, usually by culturing the cells in a fresh medium for 12 h, and were tested for suppressor activity in the in vitro secondary IgG antibody response to DNP-KLH.

Absorption and Elution of Suppressor Factor Produced by the Activated Acceptor Hybridoma with Immunoadsorbent Columns. The methods of absorption and acid elution of the suppressor factor, using protein-conjugated Sepharose 4B columns, were described previously (7). The extracted suppressor factor was used in this experiment.

Assay for TsF Activity. The assay systems to detect the suppressor activity of the absorbed or unabsorbed monoclonal KLH-TsF or the factor produced by the activated acceptor hybridoma are the same as described elsewhere (7). Briefly, 4 × 10^6 spleen cells of C57BL/6 or C3H mice primed 4 wk previously with DNP-KLH or DNP-OVA and 1 × 10^9 BPV were cultured for 5 d in 1-ml wells of a Mishell-Dutton culture plate with RPMI 1640 enriched with 10% fetal calf serum in the presence of 0.1 μg/ml of DNP-KLH or DNP-OVA. The culture supernate or cell-free extract of the activated or unactivated acceptor hybridoma or the monoclonal KLH-TsF was added to the culture at the start of cultivation. Anti-DNP IgG plaque-forming cells (PFC) were enumerated by using DNP-coupled sheep erythrocytes as described elsewhere (7).

Statistical Analysis. For in vitro assays, results were analyzed with a two-tailed Student's *t* test. The probability value of 10% was considered to be insignificant.

Results

Absorption of Monoclonal KLH-TsF with the Cloned Acceptor Hybridoma. Our previous studies (1)^2 of the cellular mechanisms of the KLH-specific suppressor system have shown that the acceptor-suppressor T cells have the ability to absorb the KLH-TsF. Therefore, the cloned acceptor hybridoma cells (34S-281) were tested for their ability to absorb the activity of the hybridoma 34S-18-derived monoclonal KLH-TsF. The monoclonal KLH-TsF was incubated with the hybridoma 34S-281 cells, BW5147, or KLH-TsF-producing hybridomas (34S-704, 34S-18) in the absence of the relevant antigen, KLH, on ice for 1 h. The data in

TABLE 1

Absorption of Monoclonal KLH-TsF with Various Cloned Hybridoma Lines

Materials added to the culture*	KLH-TsF‡ absorbed with:	Function	Anti-DNP IgG PFC/culture
None	—	—	2,220 ± 610§
KLH-TsF	Unabsorbed	—	230 ± 80
KLH-TsF	BW5147	None	390 ± 210
KLH-TsF	34S-704	TsF-producer	380 ± 270
KLH-TsF	34S-18	TsF-producer	610 ± 210
KLH-TsF	34S-281	Acceptor	2,920 ± 440

* The secretory form of monoclonal KLH-TsF obtained in ascitic fluid from hybridoma (34S-18)-bearing mice was used.

‡ 100 μl of ascitic fluid containing KLH-TsF was incubated with 1 × 10⁷ cloned cell lines on ice for 1 h. The procedures were repeated three times. The supernates were collected and were tested for their suppressor activity in the in vitro secondary anti-DNP IgG PFC response of C57BL/6 spleen cells primed with DNP-KLH.

§ Arithmetic means of PFC numbers of three cultures ± SD.

Table I show that the suppressor activity of the monoclonal KLH-TsF was completely absorbed with the hybridoma 34S-281 but not with two distinct KLH-TsF-producing hybridomas (34S-704 and 34S-18) or with BW5147 thymoma cells that are parental cells for the hybridomas. As the KLH-TsF seems to be directly absorbed by the hybridoma without the antigen-bridging interaction, it is strongly suggested that the hybridoma cells carry the receptor that recognizes the KLH-TsF.

Kinetics of the Activation of the Acceptor Hybridoma with KLH-TsF. Although the hybridoma 34S-281 was supposed to bear a receptor for the KLH-TsF, no significant biological effects on the antibody formation were obtained by the hybridoma itself. However, based on our previous data obtained in the conventional KLH-specific suppressor system (1)[2] demonstrating that the KLH-TsF acts on and activates acceptor-suppressor T cells, it is possible that the acceptor hybridoma clone established is activated after stimulation with the KLH-TsF. Therefore, the hybridoma cells were incubated with the ascitic, monoclonal KLH-TsF on ice for 1 h, extensively washed, and then cultured in a fresh medium of RPMI 1640 containing 10% fetal calf serum. The culture supernates collected at different time intervals after activation were assayed for suppressor activity in the in vitro secondary anti-DNP IgG PFC response of C57BL/6 spleen cells primed with DNP-KLH. As shown in Fig. 1, the culture supernate of the unactivated hybridoma cells showed no suppressor activity. However, the activated hybridoma cells started to produce the suppressor factor at 4 h after stimulation with the KLH-TsF. Maximal suppressive effect was observed after 12 h of activation with the KLH-TsF and continued for up to >16 d.

Evidence that the KLH-TsF Stimulates the Acceptor Hybridoma. To demonstrate that the KLH-TsF does activate the acceptor hybridoma in the resting state, the acceptor hybridoma cells were stimulated with the relevant antigen, KLH, or the monoclonal KLH-TsF, unabsorbed or absorbed with various immunoad-
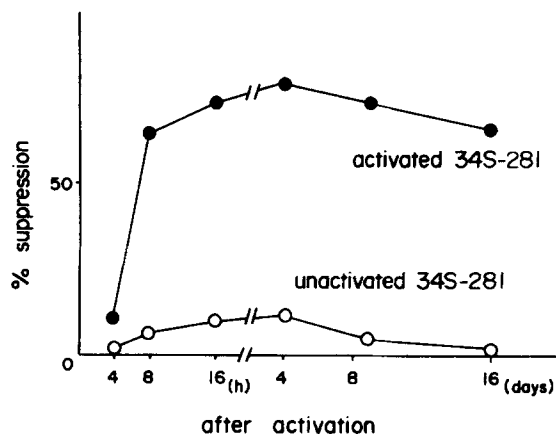
FIGURE 1.   Kinetics of the activation of the acceptor hybridoma. 1×10^7 acceptor hybridoma cells (34S-281) were incubated with 100 μl of ascites containing KLH-TsF for 1 h on ice, thoroughly washed, and then cultured in fresh RPMI 1640 enriched with 10% fetal calf serum. The supernates or the cell-free extracts of the activated or unactivated acceptor hybridoma cells were prepared by culturing cells in fresh media for 4 h, 8 h, or 16 h, and tested for their suppressor activity in the in vitro secondary anti-DNP IgG PFC response of C57BL/6 spleen cells primed with DNP-KLH. Quite similar results were obtained by stimulation with either the supernates or cell-free extracts of the activated acceptor hybridomas. The data represented here were obtained by using the culture supernates of the activated or nonactivated acceptor hybridoma and are expressed as the percent suppression of the anti-DNP IgG PFC responses compared with controls (no factor added).

sorbent columns. As shown in Table II, no suppressor effect was obtained by the incubation with KLH. Moreover, the stimulatory activity of the KLH-TsF was eliminated by absorption with immunoadsorbent columns of KLH or anti-I-J[b] but not with the rabbit anti-mouse Fab-conjugated control column. As the KLH-TsF was known to have KLH-binding moiety and I-J determinants, it is demonstrated that the KLH-TsF, in fact, works as an element to stimulate the acceptor hybridoma.

Antigen Specificity and Genetic Restriction of the Suppressor Factor Derived from the Activated Acceptor Hybridoma.   Since the activated acceptor hybridoma produced the new suppressor factor that was active in the DNP-KLH system, the antigen specificity and genetic restriction specificity of the new factor were investigated. The culture supernates or the cell-free extracts from the activated hybridoma cells were tested for their suppressor activity in the anti-DNP IgG responses of C57BL/6 (H-2[b]) mice primed with either DNP-KLH or DNP-OVA, or in those of C3H (H-2[k]) mice primed with DNP-KLH (Table III). The suppressor factor in the culture supernates or in the cell-free extracts derived from the activated acceptor hybridoma was only active in the responses of C57BL/6 mice against DNP-KLH. However, no suppressive effect was obtained in the responses of DNP-OVA-primed C57BL/6 or DNP-KLH-primed C3H mice. It is demonstrated that the newly induced factor of the activated acceptor hybridoma specifically suppresses the antibody response of syngeneic but not allogeneic recipients.

Properties of the New Suppressor Factor Derived from the Activated Acceptor Hybri-

TABLE II

Evidence that the Acceptor Hybridoma Is Stimulated by Two-Chain Type of the KLH-TsF

Acceptor hy-bridoma stimu-lated with:*	KLH-TsF[‡] absorbed with columns	Anti-DNP IgG PFC/culture
—	—	2,800 ± 500[§]
None	—	2,630 ± 450
KLH	—	2,760 ± 460
KLH-TsF	Unabsorbed	1,110 ± 540
KLH-TsF	Anti-mouse Fab	1,190 ± 250
KLH-TsF	KLH	2,860 ± 360
KLH-TsF	B10.A(5R) anti-B10.A(3R)	2,870 ± 500

* 1×10^7 acceptor hybridoma cells were incubated for 1 h on ice with 100 μg KLH or 100 μl of the extracted monoclonal KLH-TsF (equivalent to 2×10^6 hybridoma cells) that had been unabsorbed or preabsorbed with immunoadsorbent columns. They were washed and then cultured for 12 h in fresh media. The cell-free extracts (equivalent to 3×10^5 cells) of the unactivated or activated acceptor hybridomas were added to the culture and tested for their activity.

[‡] 100 μl of extracted KLH-TsF was applied to and incubated with 1 ml of immunoadsorbent columns of rabbit anti-mouse Fab, KLH, or B10.A(5R) anti-B10.A(3R) (anti-I-J[b]) for 1 h at 4°C. After 1-h incubation, the effluent from the column was collected and further reacted with acceptor hybridoma cells.

[§] Arithmetic means of PFC numbers of three cultures ± SD.

TABLE III

Specificity of the New Suppressor Factor Produced by the Activated Acceptor Hybridoma

Factor added*	Dose	Anti-DNP IgG PFC response to:		
		DNP-KLH		DNP-OVA
		C57BL/6	C3H	C57BL/6
Exp. 1				
—	—	4,420 ± 170[‡]	2,060 ± 450	1,270 ± 180
Culture supernates	200 μl	760 ± 140	1,750 ± 210	1,130 ± 210
Culture supernates	50 μl	6,490 ± 980	2,280 ± 370	1,470 ± 90
Exp. 2				
—	—	9,370 ± 1,340	1,000 ± 250	1,150 ± 120
Extracts	1×10^5	3,440 ± 190	980 ± 250	1,260 ± 420
Extracts	5×10^5	2,500 ± 1,200	930 ± 250	1,730 ± 140

* 10^7 acceptor hybridoma cells were incubated with 100 μl of the ascitic monoclonal KLH-TsF for 1 h on ice, washed, and then cultured in fresh media for 12 h. The culture supernates (50–200 μl/ml) or the cell-free extracts (equivalent to $1–5 \times 10^5$ cells) of the activated acceptor hybridoma cultured for 12 h after stimulation were added to the culture of C57BL/6 or C3H spleen cells primed with either DNP-KLH or DNP-OVA at the start of cultivation.

[‡] Arithmetic means of PFC numbers of three cultures ± SD.

doma. The data shown in Table III demonstrate that the new suppressor factor, like the KLH-TsF, shows KLH specificity and genetic restriction specificity in its action. The next experiments were designed to investigate the immunochemical properties of the newly induced suppressor factor to compare with the KLH-TsF that has KLH-binding moiety and I-J[b] determinants. The cell-free extracts

TABLE IV

Properties of the New Suppressor Factor Produced by the Activated Acceptor Hybridoma*

Factor absorbed with:	Materials added to the culture	Anti-DNP IgG PFC/culture		
		Extract	Culture supernates	
		Exp. 1	Exp. 2	Exp. 3
—	—	4,520 ± 1,720[‡]	2,900 ± 840	3,960 ± 880
None	Unabsorbed factor	190 ± 110	650 ± 430	620 ± 360
B10.A(5R) anti-B10.A(3R)	Effluent	3,410 ± 902	2,800 ± 500	—
B10.A(5R) anti-B10.A(3R)	Eluate	530 ± 530	—	470 ± 310
Rabbit anti-mouse Ig	Effluent	260 ± 90	—	—
Rabbit anti-mouse Ig	Eluate	3,510 ± 810	—	—
KLH	Effluent	500 ± 350	830 ± 770	—
KLH	Eluate	5,000 ± 690	—	3,510 ± 100

* 10^7 acceptor hybridoma cells were incubated with 100 μl of the ascitic monoclonal KLH-TsF for 1 h on ice, washed, and then cultured in fresh medium for 12 h. The culture supernates (200 μl/ml) or the cell-free extracts (equivalent to 3×10^5 cells) were applied to and incubated with immunoadsorbent columns for 1 h at 4°C. The effluent or the acid-eluate from the columns was concentrated to a final volume of 100 μl by Sephadex G-200 powder and added to the culture of C57BL/6 spleen cells primed with DNP-KLH.

‡ Arithmetic means of PFC numbers of three cultures ± SD.

of the activated acceptor hybridoma were applied to the immunoadsorbent columns of antigens or antibodies. The absorbed materials were eluted with 0.175 M glycine HCl buffer, pH 3.2, under the same conditions as described elsewhere (7), and KLH-TsF was successfully eluted from the KLH or anti-I-J[b] column. The effluent or the eluate from the column was assayed for its suppressor activity in the in vitro anti-DNP IgG antibody response. The suppressor activity of the factor from the activated acceptor hybridoma was absorbed to and eluted from the anti-I-J[b] column but not from the antigen, KLH, or anti-mouse Ig (Table IV). Therefore, the new suppressor factor, like the KLH-TsF used for activation of the acceptor hybridoma, possesses the I-J determinants detected by the B10.A(5R) anti-B10.A(3R) antiserum, but this factor, unlike the KLH-TsF, has no ability to bind to the relevant antigen, KLH.

Acceptor Hybridoma Carries Antiidiotype-like Structure for KLH-TsF. The hybridoma appears to carry a receptor for the KLH-TsF. Furthermore, the factor produced by the activated acceptor hybridoma shows the inability to bind to the KLH column, despite expressing its KLH-specific suppressor activity. Therefore, it is strongly suggested that the antiidiotype-like structure for the KLH-TsF makes up the acceptor site for KLH-TsF.

To demonstrate antiidiotype-like structure on the acceptor hybridoma, three sets of experiments were carried out. First, the acceptor hybridoma cells were activated with monoclonal KLH-TsF or C57BL/6-derived KLH- or OVA-primed thymocyte extract to investigate whether the KLH specificity of TsF is required for activation. The KLH- or OVA-primed thymocyte extract used was shown to have antigen-specific suppressor activity in the in vitro antibody responses to DNP-KLH or DNP-OVA (data not shown). As shown in Table V, the KLH-TsF from either the three different hybridoma or from C57BL/6 thymocytes successfully stimulates the cloned acceptor hybridoma cells, whereas they were not

TABLE V

Stimulation of Acceptor Hybridoma with Various Antigen-specific Suppressor Factors*

Acceptor hybri-doma stimulated with:	TsF derived from:	Anti-DNP IgG PFC/culture
—	—	2,120 ± 490[‡]
None	—	2,000 ± 580
KLH-TsF	Hybridoma (34S-18)	720 ± 600[§]
KLH-TsF	Hybridoma (34S-704)	920 ± 420[§]
KLH-TsF	Hybridoma (1L-1)	920 ± 430[§]
KLH-TsF	C57BL/6 thymocytes	760 ± 70[§]
OVA-TsF	C57BL/6 thymocytes	2,550 ± 490[∎]

* 10^7 acceptor hybridoma cells were incubated with cell-free extracts obtained from three different KLH-specific suppressor hybridomas (equivalent to 2 × 10^6) or from C57BL/6 thymocytes (equivalent to 5 × 10^7) primed twice with 200 μg of KLH or OVA. The cells incubated were washed and cultured in fresh media for 12 h. The cell-free extracts of the activated acceptor hybridoma (equivalent to 3 × 10^5 cells) were added to the culture and tested for suppressor activity in the in vitro secondary antibody response to DNP-KLH.
[‡] Arithmetic means of PFC numbers of three cultures ± SD.
[§] $P < 0.05$.
[∎] Not significant.

TABLE VI

Absorption of New Suppressor Factor with Various Suppressor Hybridoma Cells*

Factor absorbed with:	Function	Anti-DNP IgG PFC/culture
—	—	1,610 ± 490[‡]
None	—	860 ± 120[§]
Clone 34S-18	KLH-TsF producer	1,600 ± 410[∎]
Clone 34S-281	Acceptor	760 ± 210[§]

* 200 μl of culture supernatants of the activated acceptor hybridoma cells were incubated with 3 × 10^7 of two different suppressor hybridoma cells for 1 h on ice. The procedures were repeated three times. After absorption, the supernates absorbed or unabsorbed were tested for their suppressor activity in the in vitro secondary anti-DNP IgG PFC response.
[‡] Arithmetic means of PFC numbers of three cultures ± SD.
[§] $P < 0.05$.
[∎] Not significant.

activated by the OVA-primed thymocyte extracts. The results demonstrated that the KLH specificity of TsF is required for activation of the particular acceptor hybridoma cells, suggesting that the acceptor site on the hybridoma recognizes the idiotype-like determinants on the conventional and monoclonal KLH-TsF. Second, the factor of the activated acceptor hybridoma was incubated with the acceptor hybridoma or the hybridoma-producing KLH-TsF to try to absorb the suppressor activity. As shown in Table VI, the suppressor activity of the newly induced factor was specifically absorbed by the KLH-TsF-producing hybridoma, strongly suggesting that the newly produced suppressor factor bears the complementary receptor for the KLH-binding TsF. Third, we attempted to kill the
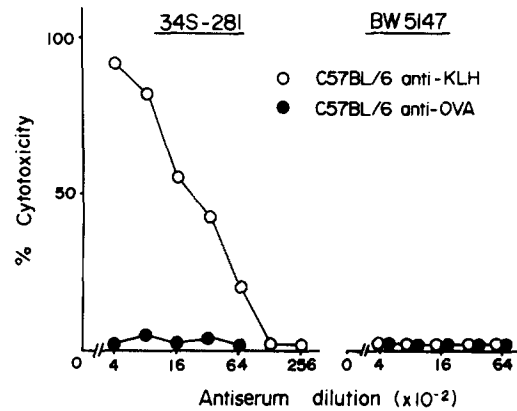
FIGURE 2. Cytotoxic curves of anti-KLH or anti-OVA antiserum on the acceptor hybridoma cells. 5×10^4 acceptor hybridoma cells or BW5147 cells were reacted with C57BL/6 anti-KLH or anti-OVA antiserum for 30 min on ice, washed, and further incubated with 1:10 diluted rabbit complement.

acceptor hybridoma cells by anti-KLH antiserum with a dye-exclusion cytotoxic assay. The hybridoma cells were reacted with anti-KLH or anti-OVA antiserum for 30 min on ice, washed, and further incubated with rabbit complement. As a control, BW5147 thymoma cells were treated under the same conditions as the experimental groups. The results are shown in Fig. 2. The acceptor hybridoma cells were killed by anti-KLH antiserum raised in C57BL/6 mice, but not by C57BL/6 anti-OVA antiserum. The cytotoxic reactivity of anti-KLH antiserum on the acceptor hybridoma was specific, because BW5147 thymoma, a parental cell line for the acceptor hybridoma, was not killed by the antiserum.

Discussion

The acceptor-suppressor T cell hybridoma obtained after fusion of KLH-primed C57BL/6 splenic T cells described in this report displayed properties distinct from other established T cell hybridomas that produce antigen-specific TsF, as follows: (a) The hybridoma 34S-281 described is a target cell for the conventional and the hybridoma-derived monoclonal KLH-TsF. Thus, the activity of the KLH-TsF is completely absorbed by the acceptor hybridoma cells (Table I). (b) The hybridoma cells are in a resting state, so that they do not express any functional activity by themselves (Fig. 1). (c) The hybridoma cells in the resting stage can, however, be directly activated by KLH-TsF and then start to produce the new factor that exerts the KLH-specific suppressor function (Fig. 1, Table II, and Table III).

Suppressor T cells of the acceptor type have been demonstrated in various suppressor systems (2–5), including our conventional KLH-specific suppressor system (1).[2] We have found that the KLH-TsF composed of two polypeptide chains of KLH-binding and I-J-positive molecules acts on and activates acceptor-suppressor T cells. In this sense, the acceptor hybridoma that was established displays similarities to the acceptor T cells of this intermediary cell type in the KLH-specific suppressor pathway.

Minami and his associates (9) have also identified an inducible suppressor T cell hybridoma obtained by the fusion of NP-primed precursors of effector-suppressor T cells. NP-specific hybridomas carried an idiotype-positive receptor for the antigen NP and were specifically activated with monoclonal antiidiotypic suppressor factor to release another NP-specific idiotype-positive suppressor factor. Minami et al. (9) have also demonstrated that the antigen NP was not required for activation of NP-specific inducible hybridoma cells. Therefore, the properties of these two NP-specific suppressor hybridomas and their factors are similar to those of our two types of KLH-specific suppressor hybridomas. The hybridoma system described here seems to represent a reciprocal pattern of the NP-specific suppressor hybridoma system.

In any event, the establishment of the hybridoma of the intermediary cell type bearing a receptor for TsF permits the analysis of the activation mechanisms in the regulatory T cell interactions and also the characterization of TsF and acceptor-suppressor T cell interaction at the molecular level. In fact, the data obtained by the experiments described in this report indicate that the acceptor hybridoma bears a receptor (acceptor) that recognizes the KLH-TsF. Since the relevant antigen, KLH, is not required for the KLH-TsF acceptor hybridoma cell interaction (Table II, Fig. 1), it is assumed that the acceptor site on the hybridoma is composed of the complementary structure for the KLH-TsF. Furthermore, the complementary structure on the acceptor hybridoma seems to be an antiidiotype-like organization for the KLH-TsF, for the following reasons: (a) The acceptor hybridoma directly interacts with KLH-TsF in the absence of antigen and is specifically activated by KLH-TsF but not by OVA-TsF. Thus, the KLH specificity of TsF is essential for activation (Table V). (b) The activated acceptor hybridoma cells start to produce monoclonal suppressor factor that displays the KLH specificity (Table III). However, the new suppressor factor obtained by the activated hybridoma did not have affinity for the antigen KLH (Table IV), whereas the KLH-TsF of the two-chain type used for stimulation of the acceptor hybridoma bound to the KLH column (7). (c) The activity of the newly induced suppressor factor was absorbed with the suppressor hybridoma that produces the KLH-TsF (Table VI). It is therefore strongly suggested that the acceptor site has a complementary structure (antiidiotype) for the KLH-TsF. Moreover, the idiotypic determinant on the KLH-TsF was found to have a structure similar to that on some of the anti-KLH antibodies, in that the acceptor hybridoma was specifically killed by the conventional anti-KLH antibodies plus complement (Fig. 2).

The above evidence demonstrates that even in a conventional antigen system, such as KLH, the antigen-binding TsF possesses a T cell idiotope that cross-reacts with some of the anti-KLH antibodies. Predominant idiotypes on anti-KLH antibodies have not so far been demonstrated in various mouse strains, including the C57BL/6 mice used in this study. Unlike NP in Igh^b mice such as C57BL/6, ABA in A/J mice, or phosphorylcholine (PC) in BALB/c mice, which raise the antibodies with major idiotypes (reviewed in 10), it is believed that the antigen KLH equally stimulates a variety of anti-KLH clones, resulting in no major idiotypes being found on the anti-KLH antibodies. Therefore, the KLH system is apparently distinct from other systems in which the antigens, like NP,

GT, ABA, or PC stimulate only specific clones in particular animals.

The KLH idiotype expressed on the KLH-TsF described may be predominant, especially at the T cell level, because conventional KLH-TsF has the ability to stimulate the particular acceptor hybridoma clone (Table V) and also because we have successfully established two distinct suppressor hybridoma clones with complementary receptors by the fusion experiment with a random selection process. Moreover, the monoclonal KLH-TsF obtained from three independent hybridoma clones 34S-18, 34S-704, and 1L-1) established by the different fusion experiments were found to stimulate the cloned acceptor hybridoma 34S-281 (Table V). Since the three independent suppressor hybridoma clones and their factors have a similar structure that is complementary to the acceptor site of the hybridoma clone 34S-281, it is quite likely that these monoclonal KLH-TsF possess the predominant idiotype. If so, the question to be asked is why the particular repertoire limitation in T cells is dictated even in the conventional antigen system. One possibility is that the family of suppressor T cells recognizes only the limited epitopes (suppressor epitope) on the conventional antigen, as measured by Sercarz and his associates (11). In that case, the suppressor T cells and their factors should bear a predominant idiotype, whereas B cells or antibodies can recognize whole epitopes on the KLH molecule. Thus, the idiotype-antiidiotype network in the conventional antigen system may operate in the interactions more effectively at T cell levels than at the T-B levels described (reviewed in 12) in various systems using particular antigens such as NP, ABA, etc. In fact, the monoclonal "idiotype-specific" TsF derived from the activated acceptor hybridoma suppressed the majority of antibody responses against total epitopes on KLH macromolecules. It is thus suggested that the monoclonal idiotype-specific TsF may effectively induce the idiotype-bearing suppressor T cells that recognize one of the epitopes of the KLH molecule by which the whole anti-KLH antibody responses are suppressed via antigen-bridging interaction. Experiments to prove these possibilities are being now undertaken.

Summary

An acceptor hybridoma with a receptor that recognizes the keyhole limpet hemocyanin (KLH)-specific suppressor T cell factor (KLH-TsF) was established after the fusion of C57BL/6 splenic T cells enriched with KLH-coated petri dishes. The cloned hybridoma (34S-281) could be specifically activated by stimulation with the conventional KLH-TsF or monoclonal KLH-TsF from three different hybridomas in the absence of the relevant antigen (KLH) and it started to produce another factor that suppresses the antibody response against DNP-KLH in a KLH-specific fashion. The KLH specificity of the TsF was required for activation. The new factor was found not to bind the KLH but to be absorbed with the KLH-TsF-producing hybridoma. It is thus strongly suggested that the acceptor site has a complementary structure (antiidiotype) for the KLH-TsF. Moreover, the idiotypic determinant on KLH-TsF was found to have a structure similar to that on some of the anti-KLH antibodies, since the acceptor hybridoma was specifically killed by the conventional anti-KLH antibodies and complement. Drawing on the above results, the idiotype-antiidiotype network in the conventional antigen system is discussed.

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