

CELLULAR CONSEQUENCES IN THE SUPPRESSION OF ANTIBODY RESPONSE BY THE ANTIGEN-SPECIFIC T-CELL FACTOR*

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Previous studies from our laboratory indicated that a soluble factor extracted from carrier-primed suppressor T cells (TsF)¹ inhibits the in vitro secondary antibody response against a hapten coupled to the same carrier. The factor was found to possess determinants controlled by a locus (Ia-4) mapped in the I-J subregion of the mouse H-2 histocompatibility complex (1). Unlike other antigen-specific TsF, there has been shown a strict genetic restriction in that TsF derived from one strain of animals can suppress the response of only H-2 histocompatible strains (2, 3).

Furthermore, TsF was shown to be absorbable by splenic T cells, but not by B cells or macrophages of the same H-2 haplotype origin. Such T cells, which were assumed to be the direct targets of TsF, were adherent to a tightly packed nylon-wool column, but were definitely killed by anti-Thy-1 antiserum (2). Thus, the suppression of the antibody response by TsF is mediated by an interaction between the TsF and the acceptor site on the target cells. The most reasonable explanation is that such an acceptor site is controlled by a gene closely linked to that for the TsF within the same H-2 complex, as there have been no exceptional cases in which H-2 histoincompatible TsF can initiate the specific suppression.

Because little is known about the consequences of this initial interaction between TsF and acceptor T cells, we have performed a series of experiments in which subsequent cellular events after the TsF-acceptor interaction were studied. In this communication, we wish to report that the final suppression of antibody response was, in fact, achieved via the intermediary type of the acceptor T cells. Some properties and the mode of action of this cell type are described.

Materials and Methods

Antigens. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. Hen's egg albumin (EA), recrystallized five times, was obtained by the method of Kekwick and Cannan (4). Dinitrophenylated KLH (DNP₇₇₀-KLH) and dinitrophenylated EA (DNP₁₂-EA) were prepared by coupling with 2,4-dinitrobenzenesulfonic acid under alkaline condition by the method of Eisen et al. (5). *Bordetella pertussis* vaccine (BPV) was purchased from the Chiba Serum Institute, Chiba, Japan.

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¹ *Abbreviations used in this paper:* BPV, *Bordetella pertussis* vaccine; C, complement; DNP, 2,4-dinitrophenyl; DNP₁₂-EA, dinitrophenylated hen's egg albumin; DNP₇₇₀-KLH, dinitrophenylated keyhole limpet hemocyanin; EA, hen's egg albumin; GAT, L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰; GT, L-glutamic acid⁶⁰-L-tyrosine⁶⁰; KLH, keyhole limpet hemocyanin; MEM, Eagle's minimum essential medium; MHC, major histocompatibility complex; TsF, suppressive T-cell factor.

Animals. Randomly bred C3H/HeJ and BALB/cAnN mice 8–12 wk old were raised in our animal facility.

Immunization of Mice. Mice were immunized intraperitoneally with 100 μ g of DNP₇₇₀-KLH or DNP₁₂-EA with 1×10^9 BPV as described previously (6). Spleen cells were obtained 4–8 wk after immunization.

Preparation of Nylon-Wool-adherent and -nonadherent Spleen Cells. Spleen cells from carrier-primed or unprimed mice were fractionated by the method of Julius et al. (7). Carrier-primed spleen cells were obtained from mice that had been immunized with 100 μ g of KLH or EA plus 1×10^9 BPV 4 wk previously. In brief, well-washed, sterile nylon wool of LP-1 Leukopak Leukocyte filters (Fenwal Inc., Walter Kidde & Co. Inc., Ashland, Mass.) was packed into a 100-ml glass syringe to the 40-ml mark. After sterilization, the column was rinsed with 200 ml of Eagle's minimum essential medium (MEM) containing 5% fetal calf serum at 37°C. The column was drained of excess medium and placed in an incubator at 37°C for 1 h before loading the cells. A total of 1×10^9 spleen cells in a vol of 20 ml was applied to the column. The column was left to stand for 45 min at 37°C, and then it was eluted slowly with warm, enriched MEM. After elution, the nylon was placed in a 1-liter beaker and was shaken in 300 ml of cold (4°C), enriched MEM to obtain nylon-wool-adherent cells. Under the conditions stated above, 15–20% of the original cells were recovered in the effluent, which was comprised of >95% Thy-1-positive cells and <5% Ig-bearing cells, of the nylon-wool column. The nylon-wool-adherent population had 60–70% Ig-bearing cells and 10–20% Thy-1-positive cells.

Preparation of Antigen-specific TsF. The method to obtain antigen-specific TsF was described in detail elsewhere (6). Briefly, mice were immunized intraperitoneally twice with 100 μ g of KLH or EA without adjuvant at 2-wk intervals. Their thymuses and spleen cells were obtained and subsequently disrupted by sonication using a Tomy UR 150 sonicator (Tomy Seiko Co., Ltd., Tokyo, Japan). The cell-free supernate was obtained by ultracentrifugation at 40,000 *g* for 1 h.

Alloantisera. Anti-Ia antisera were supplied by Dr. C. S. David, Mayo Medical School, Rochester, Minn. The combination of the mouse strains to produce these antisera and resultant specificities were shown in the Results. Anti-Thy-1.2 antisera (AKR anti-C3H) were raised in our laboratory.

Treatment of Cells with Alloantisera. 0.5 ml of 1:5 (anti-Ia) or a 1:10 (anti-Thy-1.2) dilution of alloantisera was added to 0.5 ml of cell suspension containing 1.5×10^7 viable spleen cells. They were incubated at 0°C for 45 min, washed twice with cold MEM, and were then treated with a well-selected rabbit or guinea pig complement (C) diluted to 1:10 at 37°C for 30 min.

Cell Cultures. The modified Marbrook culture system was utilized. 5×10^6 DNP-primed B cells obtained from DNP₇₇₀-KLH-primed spleen cells treated with anti-Thy-1.2 plus C were cocultured with an equal number of carrier-primed nylon-wool-purified helper T cells with or without the addition of 2.5×10^6 nylon-wool-adherent cells. They were incubated for 5 d with 0.1 μ g/ml of appropriate antigen at 37°C in 10% CO₂. The number of DNP-specific IgM and IgG plaque-forming cells were assayed using DNP-coupled sheep erythrocytes as described previously (6).

Results

Requirement of Antigen-primed Nylon-Wool-adherent T Cells for the Suppression by TsF. The DNP-specific in vitro secondary IgG antibody response was effectively induced by cocultivation of DNP-primed B cells with nylon-wool purified KLH-primed helper T cells, whereas low IgG response was elicited with KLH-primed nylon-wool-adherent T cells (Table I). However, the addition of KLH-specific TsF did not significantly suppress the response mounted by B cells and nylon-wool-purified helper T cells.

This suggests that a third cell type presumably present in the adherent population is required for the effect of TsF. To prove this postulate, 2.5×10^6 adherent cells were added to the culture of B and helper T cells. This dose of adherent cells did not affect the net IgG antibody response. However, the addition of TsF into this mixture greatly suppressed the antibody response (Table I).

TABLE I
Requirement of Nylon-Wool-adherent Cells for the Suppression Induced by TsF

Cell mixture‡			KLH-TsF added§	Anti-DNP IgG PFC/culture
DNP-primed B cells*	Nylon-wool-purified T cells	Nylon-wool-adherent cells¶		
$\times 10^6$	$\times 10^6$	$\times 10^6$		
5	5	—	—	2,231 \pm 245**
5	5	—	+	2,345 \pm 350
5	—	5	—	231 \pm 23
5	—	5	+	261 \pm 70
5	5	2.5	—	2,176 \pm 354
5	5	2.5	+	681 \pm 151
5	—	—	—	11 \pm 4

* DNP-KLH-primed spleen cells treated with anti-Thy-1.2 and guinea pig C.

‡ 5×10^6 DNP-primed B cells from C3H spleen cells were cocultured with 5×10^6 nylon-wool-purified helper T cells and/or $2.5\text{--}5.0 \times 10^6$ nylon-wool-adherent cells from KLH-primed C3H spleen cells in the presence of $0.1 \mu\text{g}$ DNP-KLH.

§ KLH-TsF from C3H mice corresponding to 1×10^7 viable spleen cells was added at the start of cultivation.

¶ KLH-primed splenic cells passed through the tightly packed nylon-wool column.

|| KLH-primed splenic cells adherent to the tightly packed nylon-wool column.

** Arithmetic means and standard deviations from five cultures.

TABLE II
Necessity for the Carrier Specificity of Nylon-Wool-adherent Cells in the Induction of the Effective Suppression

Nylon-wool-purified T cells*	Nylon-wool-adherent cells‡	Stimulating antigen	TsF added§	Anti-DNP IgG PFC/culture
KLH	—	DNP-KLH	—	2,448 \pm 68
KLH	KLH	DNP-KLH	—	2,535 \pm 898
KLH	KLH	DNP-KLH	KLH	620 \pm 240
KLH	KLH	DNP-KLH	EA	1,798 \pm 557
KLH	Unprimed	DNP-KLH	—	1,077 \pm 468
KLH	Unprimed	DNP-KLH	KLH	908 \pm 312
KLH	Unprimed	DNP-KLH	EA	1,101 \pm 165

* 5×10^6 DNP-primed B cells from C3H spleen cells treated with anti-Thy-1.2 + C were cocultured with 5×10^6 nylon-wool-purified helper T cells and 2.5×10^6 nylon-wool-adherent cells from primed or unprimed C3H spleen cells with or without KLH- or EA-TsF.

‡ 2.5×10^6 nylon-wool-adherent splenic cells from KLH-primed mice.

§ KLH- or EA-TsF from C3H mice corresponding to 1×10^7 viable cells was added at the start of cultivation.

|| Arithmetic means and standard deviations from five cultures.

The question arises as to whether or not the adherent cells have to be primed with relevant antigen. Thus adherent cells were taken either from KLH-primed or unprimed animals and then added to the mixture of B cells and KLH-primed helper T cells. As shown in Table II, KLH-specific TsF was able to induce suppression in the presence of KLH-primed nylon-wool-adherent cells, whereas normal cells were incapable of inducing the suppressive effect. The observed suppression was found to be antigen-specific, because EA-specific TsF did not suppress the response in the presence of KLH-primed adherent cells.

TABLE III
Evidence that a Nylon-Wool-adherent Cell is the Target of TsF

Culture*			Anti-DNP IgG PFC/culture
Cells‡	Cell number	Treatment§	
Nylon purified +	5×10^6	—	2,405 ± 117
Nylon adherent Nylon purified +	2.5×10^6 5×10^6	— KLH-TsF	
Nylon adherent Nylon purified +	2.5×10^6 5×10^6	— —	2,408 ± 203
Nylon adherent	2.5×10^6	KLH-TsF	
—	—	—	10 ± 5

* 5×10^6 DNP-primed B cells from C3H spleen cells treated with anti-Thy-1.2 and C were cocultured with 5×10^6 KLH-primed nylon-wool-purified helper T cells and 2.5×10^6 nylon-wool-adherent cells, either of which had been pretreated with KLH-TsF at 0°C for 1 h before the culture.

‡ KLH-primed spleen cells passed through or adherent to nylon-wool columns were treated with KLH-TsF at 0°C for 1 h. They were then washed and added to the culture.

§ KLH-TsF from C3H mice corresponding to 1×10^7 viable cells.

|| Arithmetic means and standard deviations from five cultures.

Evidence that Nylon-Wool-adherent T cells are the Direct Target of the Antigen-specific TsF. To confirm that the nylon-wool-adherent cell is the target of TsF, the adherent and nonadherent populations from KLH-primed mice were incubated with KLH-specific TsF at 0°C for 1 h without antigen. Cells were then washed twice with cold MEM and were cocultured with DNP-primed B cells in the presence of DNP-KLH. As shown in Table III, the addition of nylon-wool-adherent cells pretreated with KLH-specific TsF significantly suppressed the response mounted by DNP-primed B cells and nylon-wool-purified helper T cells. The same treatment of nylon-wool-purified helper T cells did not produce any suppressive effect. Because the nylon-wool-adherent population contains mostly B cells and macrophages, but only a small number of T cells, we attempted to define the type of cells which mediated this suppression. KLH-primed nylon-wool-adherent cells were treated with anti-Thy-1.2 and guinea pig C. 2.5×10^6 treated or nontreated nylon-wool-adherent cells were added to the coculture of DNP-primed B cells and KLH-primed nylon-wool-purified helper T cells with or without TsF. As shown in Table IV, the addition of nylon-wool-adherent cells together with TsF gave strong suppression. This suppression was, however, completely abrogated by the treatment of nylon-wool-adherent cells with a higher dilution of anti-Thy-1.2 and C. The same effect was obtained by the treatment with lower dilution of anti-Thy-1 and C. The results suggest that a high density of Thy-1 antigen is present on nylon-wool-adherent T cells involved in this suppression. The treatment with higher concentration of anti-Thy-1.2 was, however, found to eliminate both target cells for TsF and helper T cells (Th₂) present in the nylon-wool-adherent cell population which can polyclonally help the B-cell response as described by Tada et al. (8).

Presence of the I-J Subregion Gene Product on the Acceptor Site for TsF. KLH-primed

TABLE IV
Abolishment of the TsF-mediated Suppressive Activity by the Pretreatment of Nylon-Wool-adherent Cells with Anti-Thy-1.2 and C

Adherent cells treated with*	Dilution of antiserum	TsF added‡	Anti-DNP IgG/culture
C§	—	—	1,277 ± 259
C	—	+	57 ± 17
Anti-Thy 1.2 + C	1:20	—	526 ± 110
Anti-Thy 1.2 + C	1:20	+	437 ± 201
Anti-Thy 1.2 + C	1:60	—	1,279 ± 126
Anti-Thy 1.2 + C	1:60	+	1,291 ± 302

* C3H nylon-wool-adherent cells were treated with anti-Thy-1.2 and C before the culture. They were then washed thoroughly with medium. 2.5×10^6 of the cells were cocultured with DNP-primed B cells and KLH-primed nylon-wool-purified helper T cells from C3H mice with or without KLH-TsF in the presence of 0.1 µg DNP-KLH.

‡ KLH-TsH from C3H mice corresponding to 1×10^7 viable cells was added to the culture.

§ Guinea pig C.

|| Arithmetic means and standard deviations from five cultures.

TABLE V
The Presence of I-J Subregion Gene Product on the Acceptor Cells for TsF

Adherent cells treated with*	Subregion specificity	Comple-ment‡	TsF added§	Anti-DNP-IgG PFC/culture
—	—	—	—	1,290 ± 198
A.TL anti-A.TH	—	+	—	1,170 ± 236
A.TL anti-A.TH	—	—	+	250 ± 88
		+	+	61 ± 26
A.TH anti-A.TL	A,B,J,E,C	—	+	1,631 ± 578
		+	+	1,344 ± 178
(C3H.Q × B10.D2)F ₁ anti-AQR	A,B,J,E	—	+	1,058 ± 206
		+	+	942 ± 286
(A.TH × B10.A(5R))F ₁ anti-A.TL	A,B,C	—	+	145 ± 106
		+	+	50 ± 27
B10.A (3R) anti-B10.A(5R)	J	—	+	1,062 ± 58
		+	+	1,612 ± 361
B10.S (7R) anti-B10.HTT	E,C	—	+	142 ± 114
		+	+	93 ± 41

* KLH-primed nylon-wool-adherent cells from C3H mice were treated with various alloantisera with or without C. They were then washed twice with cold MEM, and 2.5×10^6 of the cells were cocultured with 5×10^6 DNP-primed B cells and 5×10^6 KLH-primed nylon-wool-purified helper T cells from C3H mice in the presence or absence of KLH-TsF.

‡ Rabbit C.

§ KLH-TsF from C3H mice corresponding to 1×10^7 viable cells was added at a start of cultivation.

|| Arithmetic means and standard deviations from five cultures.

nylon-wool-adherent cells were treated with various alloantisera with different I-subregion specificities. The treatment was performed with or without rabbit C. As shown in Table V, the suppressive effect by KLH-specific TsF was completely abrogated by the treatment with anti-Ia antisera having specificity for products of the I-J subregion, regardless of other subregion specificities. The treatment with those lacking I-J subregion specificity did not affect the function of the acceptor T cells. Table V, also includes the results of the treatment of nylon-wool-adherent cells with

TABLE VI
Induction of Suppressor T Cells by the Precultivation of Nylon-Wool-adherent Cells with TsF and Antigen

Preculture*			Precultured cells treated with‡	Anti-DNP IgG PFC/culture
Adherent cells	Antigen	KLH-TsF		
+	-	+	-	942 ± 69§
+	KLH	-	-	1,495 ± 400
+	KLH	+	-	231 ± 85
+	KLH	+	Anti-Thy-1.2 + C	1,207 ± 249

* 1×10^7 /ml KLH-primed nylon-wool-adherent cells from C3H mice were precultured with or without 0.1 μ g KLH and/or KLH-TsF for 48 h in the Marbrook bottle. They were then harvested, washed thoroughly with MEM, and 2.5×10^6 of the cells were further cocultured for 5 d with DNP-primed B cells and KLH-primed helper T cells without KLH-TsF in the presence of 0.1 μ g DNP-KLH.

‡ KLH-primed nylon-wool-adherent cells precultured with 0.1 μ g KLH and KLH-TsF for 48 h were treated with anti-Thy-1.2 and C before further cultivation with DNP-primed B cells and KLH-primed helper T cells.

§ Arithmetic means and standard deviations from five cultures.

anti-Ia for 1 h at 0°C without C. The intermediary function of the acceptor T cells was completely blocked by the relevant antisera having anti-I-J activity.

Induction of Antigen-nonspecific Suppressor T Cells From Nylon-Wool-adherent Cell Population in the Presence of TsF. To understand the properties of nylon-wool-adherent cells after accepting TsF, KLH-primed adherent cells were cultured with TsF in the presence of 0.1 μ g KLH for 48 h. Cells were then washed thoroughly with medium and added to the culture of DNP-primed B cells and KLH-primed helper T cells. In some experiments, the cultured adherent cells were treated with anti-Thy-1.2 and C. The results shown in Table VI, clearly demonstrate that the addition of the adherent cells precultured with TsF and antigen caused strong suppression, whereas the suppressive activity was abrogated by the treatment of the cells with anti-Thy-1.2 and C. These results indicate that TsF generates new suppressor T cells from the KLH-primed nylon-wool-adherent cells in the presence of antigen during the 48-h culture.

The antigen specificity of newly induced suppressor T cells by TsF was investigated. The mixture of DNP-primed B cells and EA-primed nylon-wool-purified helper T cells was cultured with KLH-primed nylon adherent cells together with KLH-specific or EA-specific TsF in the presence of 0.1 μ g DNP-EA and 0.1 μ g free KLH. Under these conditions, DNP-primed B cells and EA-primed helper T cells effectively cooperated to induce a secondary anti-DNP IgG response. The response against DNP-EA was, however, suppressed by KLH-specific TsF, but not by EA-specific TsF when the KLH-primed adherent cells coexisted (Table VII, upper part). The same conclusion was reached from the reverse experiments as illustrated in the lower part of Table VII. The suppression by EA-specific TsF was induced only in the presence of EA-primed but not KLH-primed nylon-wool-adherent cells. The results imply that KLH-primed nylon-wool-adherent T cells after accepting KLH-specific TsF in the presence of the relevant free carrier (KLH) can suppress the response against unrelated antigen (DNP-EA) even though TsF is not specific for the relevant (EA) carrier to the helper T cells; furthermore, the identity of the antigen specificity between TsF and adherent T cells is required.

Non-Requirement of H-2 Histocompatibility between Acceptor T Cells and Helper T Cells for

TABLE VII
Requirement for the Identity of Antigen Specificity between Adherent Cells and TsF

Nylon-wool-purified T cells primed with	Nylon-wool-adherent cells primed with	Stimulating antigens	TsF added*	Anti-DNP IgG PFC/culture
EA	KLH	DNP-EA + KLH	—	1,259 ± 136‡
EA	KLH	DNP-EA + KLH	KLH	310 ± 120
EA	KLH	DNP-EA + KLH	EA	1,203 ± 88
KLH	EA	DNP-KLH + EA	—	1,848 ± 320
KLH	EA	DNP-KLH + EA	EA	260 ± 31
KLH	EA	DNP-KLH + EA	KLH	1,623 ± 187

5×10^6 DNP-primed BALB/c B cells were admixed with 5×10^6 nylon-wool-purified helper T cells and 2.5×10^6 nylon-wool-adherent cells from BALB/c mice primed with indicated antigen (KLH or EA). The mixture was cultured with either KLH-TsF or EA-TsF in the presence of 0.1 μ g DNP-EA and KLH, or 0.1 μ g DNP-KLH and EA.

* KLH- or EA-TsF from BALB/c mice corresponding to 1×10^7 viable cells was added at the start of cultivation.

‡ Arithmetic means and standard deviations from five cultures.

TABLE VIII
Non-Requirement of H-2 Compatibility between Induced Suppressor T Cells and Helper T Cells for the Induction of Suppression

Nylon-wool-purified helper T cells from	Adherent cells from	TsF from*	Anti-DNP IgG PFC/culture
C3H	—	—	1,155 ± 142‡
C3H	C3H	—	3,343 ± 103
C3H	C3H	C3H	462 ± 138
C3H	C3H	BALB/c	3,119 ± 77
C3H	BALB/c	—	1,126 ± 143
C3H	BALB/c	C3H	992 ± 24
C3H	BALB/c	BALB/c	145 ± 77

2.5×10^6 KLH-primed nylon-wool-adherent cells either from C3H or BALB/c mice were added to the mixture of 5×10^6 DNP-primed B cells and KLH-primed nylon-wool-purified helper T cells from C3H mice. They were cultured for 5 d with KLH-TsF either from C3H or BALB/c mice in the presence of 0.1 μ g DNP-KLH.

* KLH-TsF either from C3H or BALB/c corresponding to 1×10^7 viable cells was added at a start of cultivation.

‡ Arithmetic means and standard deviations from five cultures.

the TsF-mediated Suppression. The requirement of histocompatibility was investigated in the suppressive interaction between acceptor T cells, helper T cells, and TsF. DNP-primed B cells and nylon-wool-passed KLH-primed helper T cells from C3H mice were cocultured with KLH-primed syngeneic (C3H) or allogeneic (BALB/c) adherent cells in the presence of KLH-specific C3H or BALB/c TsF. As shown in Table VIII, the response of the mixture of C3H B cells, nylon-wool-passed helper T cells, and adherent cells was suppressed by C3H TsF but not by BALB/c TsF. BALB/c TsF, however, suppressed the response of C3H B cells and nylon-wool-purified helper T cells only if BALB/c nylon-wool-adherent cells coexisted. This suppressive effect mediated by BALB/c nylon-wool-adherent cells and BALB/c TsF was obviously not a result of the allogeneic effect, because no effect was obtained when BALB/c nylon-

wool-adherent cells were added to the culture of C3H B cells and nylon-wool-purified helper T cells together with C3H TsF.

Discussion

A certain mechanism of action of the antigen-specific TsF was studied in the secondary antibody response. Antigen-specific TsF acts on the nylon-wool-adherent T cells which have acceptor sites for TsF, generating an actual effector type of suppressor T cells.

Our previous studies have shown that TsF can be absorbed with syngeneic adherent T cells to the nylon-wool column, but not with B cells, macrophages, nylon-wool-purified T cells or allogeneic T cells, whereas the final target cells for TsF were found to be helper T cells (2). We have indeed been able to demonstrate that the suppressive effect of TsF was mediated only if the KLH-specific nylon-wool-adherent cells were present in the culture of DNP-primed B cells and KLH-primed helper T cells (Tables I and II). This suggested a intermediary function of nylon-wool-adherent cells in the suppressor cell interaction. The adherent cells involved in this suppression were shown to be T cells expressing the high density of Thy-1 antigen, because the effect was abolished by the treatment of the cells with a higher dilution of anti-Thy-1.2 and C (Table IV). The primed state of the adherent cells was demonstrated to be necessary for TsF-acceptor cell interaction (Table III and VII). Thus, the direct target cell possessing the acceptor site for TsF is a primed T cell adherent to the nylon-wool column.

The intermediary function of the acceptor T cell was completely abrogated by the cytotoxic treatment with alloantibodies that had specificity to the product of genes in the I-J subregion of the MHC, but not by those of antibodies lacking this I-J specificity (Table V). More specifically, the function was blocked by the treatment with anti-I-J antiserum in the absence of C. This strongly suggests that the acceptor site for TsF is coded for by genes in the I-J subregion, although the possibility that anti-I-J antibodies sterically interfere with TsF binding to the acceptor site, which is non-I-J-products in nature, is still not excluded. These results, therefore, well explain that the requirement of strict genetic restriction, in which identities exist among genes in the I-J subregion of the MHC between the donor of the factor and the acceptor cells, is necessary for effective suppression (2, 3).

KLH-primed adherent cells precultured with KLH-specific TsF plus antigen (KLH) for 48 h became strongly suppressive when they were added to the mixture of B cells and nylon-wool-purified helper T cells. The suppressive activity was abolished by the treatment of precultured cells with anti-Thy-1.2 and C before the addition to the culture (Table VI). Thus it is apparent that TsF activates acceptor cells to generate new suppressor T cells, acting as an actual effector cell type.

The question now to be asked is whether the induced suppressor T cells in our system have the same properties as the producer of TsF. Table VII shows the antigen specificity of induced suppressor T cells. KLH-primed nylon-wool-adherent T cells, after acceptance of KLH-specific but not EA-specific TsF, suppressed the response to DNP-EA mounted by DNP-primed B cell and EA-primed helper T cells. In other words, antigen-nonspecific suppression induced by KLH-TsF was mediated only when KLH-primed acceptor T cells and specific antigen (KLH) were both present in the response to DNP-EA. Therefore, identity of antigen specificity between TsF and

acceptor T cells is required in the induction of this nonspecific suppression. Furthermore, adherent cells, after acceptance of syngeneic but not allogeneic TsF, could suppress both syngeneic and allogeneic antibody responses (Table VIII). The identities among MHC genes between TsF and acceptor T cells are definitely required for the induction of the effector type of suppressor T cells, whereas the final step of suppression can be induced by either syngeneic or allogeneic induced suppressor T cells. These findings imply that there are amplification loops in the regulatory pathways by which initial antigen-specific, genetically restricted regulatory forces are transformed into antigen-nonspecific effects that are exerted across the H-2 barrier.

Taken collectively, it seems that at least two distinct T cells are involved in the suppression of antibody response, one of which produces antigen-specific TsF, and the second of which is activated by this TsF plus antigen to become the effector type of suppressor T cells. The experiments of Feldmann and Kontiainen (9) on T₁-T₂ cell interaction in the antibody suppression support this concept. This is also supported by the experiments of Germain et al. (10), Waltenbaugh et al. (11) and Kapp (12) on the induction of antigen-specific suppressor T cells by L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT)-TsF or L-glutamic acid⁵⁰-L-tyrosine⁵⁰ (GT)-TsF. Germain et al. (10) have shown that the addition of GAT-TsF to the culture of syngeneic spleen cells stimulates the development of GAT-specific suppressor T cells. Waltenbaugh et al. (11) and Kapp (12) have also demonstrated that suppressor cells induced by GAT-TsF or GT-TsF in naive mice can inhibit the primary anti-GAT or anti-GT response in an antigen-specific fashion.

Genetic restrictions of cell interaction have been reported in several experimental systems (13-17). In the suppressor cell interaction, the genetic restrictions were mapped in the I-J subregion of MHC (2). GAT-TsF and GT-TsF, however, act across H-2 barriers in the suppressor T cell induction (10-12). Kontiainen and Feldmann (18) have also demonstrated that antigen-specific suppressor factor produced by metabolically active, *in vitro* induced suppressor T cells inhibits both syngeneic and allogeneic antibody responses. These findings are partially in contrast to our results. Their factors were mostly assayed on the *in vitro* and *in vivo* primary responses, whereas KLH-TsF was assayed on secondary response. It is, therefore, possible that the discrepancy between KLH-TsF and other factors is a result of the primed state of the target cells.

The genetic restriction in the suppressor cell interaction must be considered in relation to the biological role of I-J determinants on suppressor molecules, because I-J products have been shown to be present on suppressor T cells and their factors in various systems (2, 3, 10-12, 18). Recent studies from our laboratory on T-cell hybridomas with suppressor function have shown that the antigen-specific suppressor molecule is composed of at least two distinct molecules; antigen-binding and I-J-bearing molecules, and the association of which is required for the antigen-specific I-J-restricted suppressive cell interaction (19, 20). Furthermore, I-J subregion gene products were expressed on T-cell hybridomas and their factors with different functional activities, e.g., antigen-specific, antigen-nonspecific, and no suppressive function (19, 21). I-J products on the antigen-specific T-cell hybridoma with restriction specificity was found to be serologically different from that on the T-cell hybridoma with nonspecific, genetically nonrestricted suppressor function (22). Therefore, heterogeneity of I-J subregion gene products may reflect functional difference between

subsets of I-J-bearing suppressor T cells. The potential roles of I-J subregion gene products on the suppressor T cells and their factors can be explored by using I-J positive T-cell hybridomas with different functional specificities.

Although the actual cellular events are still largely unknown, the suppressive mechanism is indeed maintained and amplified by the presence of the novel type of T cells. The close characterization of the intermediary type of T cells and the use of I-J-bearing T-cell hybridomas may lead us to understand the interrelationship between various T-cell factors.

Summary

Cellular events mediated by antigen-specific soluble factor extracted from carrier-primed suppressor T cells (TsF) in the suppressive interaction was studied. Keyhole limpet hemocyanin (KLH)-specific TsF directly acts on KLH-primed, I-J positive, nylon-wool-adherent T cells that have an acceptor site for TsF. The nylon-wool-adherent T cells, after accepting TsF in the presence of specific antigen, generate new suppressor T cells acting as an actual effector cell type. Antigen-specificity and syngeneity at I-J between TsF and acceptor T cells are both required for the induction of new suppressor T cells. Newly induced suppressor T cells, however, suppress both syngeneic and allogeneic responses in an antigen-nonspecific fashion.

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