MONOCLONAL SUPPRESSOR FACTOR SPECIFIC FOR LACTATE DEHYDROGENASE B

I. Mechanism of Interaction Between the Factor and Its Target Cells*

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Antigen-specific, T cell-derived factors have been the subject of extensive studies as possible mediators of immunoregulatory interactions (reviewed in references 1–3). Functionally, two kinds of factor, helper and suppressor, can be distinguished. The suppressor factors (TsF),[1] again, fall into two categories, namely, inducer factors (TsiF) that play a role in communication between T cell sets of the suppressor pathway, and effector factors (TseF) that cause ultimate suppression. Because the immune response to a single antigen may involve a multitude of regulatory factors, it is mandatory to establish a pure source of these mediators for further biological and biochemical studies. To this end, several laboratories have produced T cell lines or T cell hybridomas that secrete monoclonal TsF. While the role of some of these TsF in the suppressor pathway has not yet been determined (4–6), other factors have been shown to possess TsiF (7, 8) or TseF (9–13) activity. Thus, there exists now a battery of monoclonal TsF suitable to study cellular interactions in different suppressor pathways. However, there are other obstacles to these studies, namely, that the cells participating in most known suppressor pathways have not been identified, and the effector functions used as a readout of suppression themselves involve complex cellular interactions (4–6, 9–14).

We have characterized recently a suppressor pathway that regulates the immune response to lactate dehydrogenase B (LDH_B) (15–17). The suppressor effector (Tse) cell in this system is an Lyt-1^+2^+ cell that becomes activated by the recognition of LDH_B together with E^k ($E^k_\alpha E^k_\beta$) molecules of antigen-presenting cells (APC) and by an additional, nonspecific, Tsi cell-derived signal. The target of the Tse cell is a proliferating Lyt-1^+2^- cell that recognizes LDH_B together with the A ($A_\alpha A_\beta$) molecules of the APC. The latter cells probably include the LDH_B-specific T helper (Th) cells, since the strain distribution of responsiveness to LDH_B is identical in terms of both T

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[1] *Abbreviations used in this paper:* A molecule, a molecule controlled by the A_α and A_β loci in the *I-A* region of *H-2*; APC, antigen-presenting cell; CFA, complete Freund's adjuvant; Con A, concanavalin A; E molecule, a molecule controlled by the E_α and E_β loci; FCS, fetal calf serum; GAT, poly($glu^{60}ala^{30}tyr^{10}$); KLH, keyhole limpet hemocyanine; LDH_B, lactate dehydrogenase B; Mhc, major histocompatibility complex; S.I., stimulation index; TCGF, T cell growth factor; Th, T helper (cell); Tse, T suppressor effector (cell); TseF, T suppressor effector factor TsF, T suppressor factor; Tsi, T suppressor inducer (cell); TsiF, T suppressor inducer factor.

cell proliferation and antibody production (15, 18). The interaction between Tse and Th is antigen-specific and major histocompatibility complex (Mhc)-restricted, and the suppression is manifested in the inhibition of the LDH_B-specific, A-restricted proliferation of the Th cells. Because it is so well characterized, the anti-LDH_B response provides an ideal system to study the mechanism of Tse–Th cell interaction.

We report here the functional characterization of monoclonal TseF secreted constitutively by T cell hybridomas. The latter were produced by fusing a long-term, LDH_B-specific Tse cell line with the BW5147 thymoma. We use this factor to clarify the mechanism of Tse–Th cell interaction.

Materials and Methods

Mice. 8–15-wk-old male and female mice were obtained from our colony at the Max Planck Institute for Biology. The strains and their alleles at *H-2* loci are listed in Tables IV and VI.

Antigens and Immunization. LDH_B (LDH-H4 from pig heart; Boehringer, Mannheim, F.R.G.), keyhole limpet hemocyanin (KLH; Calbiochem, Giessen, F.R.G.), poly($glu^{60}ala^{30}tyr^{10}$) (GAT; Miles-Yeda, Rehovoth, Israel) and UPC 10 (purified IgG_{2a} myeloma protein; Bionetics, Fresenius, Stuttgart, F.R.G.) were emulsified at 1/1 (vol/vol) ratio with complete Freund's adjuvant (CFA; Difco Hedinger, Stuttgart, F.R.G.), and 0.05 ml of the emulsion, containing 0.05 mg of antigen, was injected into mice subcutaneously, at the tail base. Concanavalin A (Con A) was purchased from Deutsche Wellcome (Burgwedel, F.R.G.).

Monoclonal Antibodies. Monoclonal A- and E-specific antibodies secreted by hybridomas B15-124R1 (anti-Ia.m2), 13/4 (anti-Ia.m7; reference 19; the cell lines were obtained from Dr. G. J. Hämmerling, German Cancer Research Center, Heidelberg, F.R.G.), 10.-2.16 (anti-Ia.m17; reference 20; obtained from the Salk Institute, San Diego, CA), Y17 (anti-Ia.m44; reference 24; a gift from Dr. D. B. Murphy, Yale University School of Medicine Dept. of Pathology, New Haven, CT), and 25-9-17 (reacts with an unassigned A^b determinant; reference 22; the hybridoma was obtained from Dr. D. H. Sachs, Transplantation Biology Section, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) were used in ascites form. Monoclonal antibodies 1L9, 2L2, and 14P, specific for a molecule controlled by a locus in the A_α-E_β interval and selectively expressed by T cells, were produced as described previously (23).

Production of Hybridomas Secreting LDH_B-Specific TseF. Long-term Tse cell line was established from LDH_B-immune lymph node-cells of B10.A(2R) (suppressed nonresponder) mice. The Lyt-1^+2^+ set containing LDH_B-specific Tse was separated by positive selection of Lyt-1^+2^+ and Lyt-1^-2^+ cells with a monoclonal Lyt-2.2 specific antibody followed by the selection of Lyt-1^+2^+ cells with a monoclonal Lyt-1.2 specific antibody (17), and the cells (1×10^5/ml) were cultured in Alpha minimal essential medium (Gibco, BCK Biocult-Chemie, Karlsruhe, F.R.G.) supplemented with 10% FCS, antibiotics, L-glutamine, and 2-mercaptoethanol, in the presence of 15 μg/ml LDH_B and syngeneic, irradiated (3,300 R) spleen cells (2.5×10^6/ml) as feeder cells, for 1 wk, followed by another week of culture in the presence of antigen, feeder cells, and T cell growth factor (TCGF; 48 h supernate of Con A-stimulated mouse spleen cells). After three such culture cycles, the cells were expanded by weekly restimulations with LDH_B, TCGF, and feeder cells. The cultured cells were found to suppress the LDH_B-specific proliferation of Lyt-1^+2^- cells in an antigen-specific and Mhc-restricted fashion. Cells of the 4-mo-old Tse line were fused with the BW5147 thymoma, and the hybridomas were selected using standard methods (4, 24). The hybridoma supernates were screened for suppression of the antigen-specific proliferation of LDH_B-primed syngeneic Lyt-1^+2^- cells. Six hybridomas (2RL-Ts-1 through 6) were found to secrete, in the absence of antigen, a suppressive material. Two of these hybridomas (2RL-Ts-1 and 5) were recloned (at a density of 0.3 cell/well). All clones tested were positive, and secreted TsF that was functionally indistinguishable from that produced by uncloned lines. Therefore, in the experiments described here, supernates of cloned and uncloned hybridomas were used indiscriminately.

Testing the Effect of TseF on Antigen-specific T Cell Proliferation. Immune lymph-node cells or the Lyt-1^+2^- fraction thereof (obtained by separation on anti-mouse IgG-coated dishes after

TABLE I

Antigen-Specificity of TseF Produced by Hybridoma Cells

Source of supernatant*	Anti-LDH_B response‡ (B10.AL, Lyt-1^+2^-)		Anti-LDH_B response B10.A(2R), Lyt-1^+2^-		Anti-KLH response‡ B10.A(2R), unseparated		Anti-GAT response‡ B10.A(2R), unseparated	
	Δ cpm	S.I.	Δ cpm	S.I.	Δ cpm	S.I.	Δ cpm	S.I.
BW5147	80,669	20.0.	55,338	13.8	14,002	6.2	8,521	4.3
2RL-Ts-1	936	1.2	567	1.1	15,278	6.6	9,034	4.7
2RL-Ts-2	933	1.2	3,735	1.3	20,651	6.5	12,025	4.0
2RL-Ts-3	526	1.1	3,722	1.2	20,172	6.8	10,676	4.1
2RL-Ts-4	−268	1.0	1,498	1.1	20,319	5.3	12,994	4.5
2RL-Ts-5	2,134	1.4	457	1.0	19,036	6.6	11,605	5.0
2RL-Ts-6	−406	1.0	1,987	1.1	21,045	5.2	11,934	3.8

* Hybridoma supernates were added to cultures at a 1:4 final dilution.

‡ Proliferation of 4×10^5 primed T cells in the presence of 15 μg/ml of LDH_B, 10 μg/ml of KLH, or 50 μg/ml of GAT.

treatment with Lyt-2.2-specific antibody, as described in reference 17) were tested for antigen-specific response in a standard 3-d proliferation assay (25). Different dilutions of the TseF or of a control factor (supernate of the BW5147 line) were included in the same culture volume (0.2 ml) and were present throughout the culture. Alternatively, the immune Lyt-1^+2^- cells were preincubated with factors at predetermined dilutions at 37°C for 6 h, the cells were then washed and tested for proliferation in the 3-d assay. The cultures were set up in triplicate, and the results were expressed as Δ cpm (the difference of cpm in cultures with and without antigen) and as stimulation index (S.I.; the ratio of cpm in cultures with and without antigen).

Absorption of TseF. 1-ml aliquots of hybridoma supernatant diluted to 1:4 or 1:10 with culture medium, were incubated for 1 h on ice with $2–4 \times 10^7$ cells using different cell populations as shown in Table IV. The cells were then centrifuged and the supernates were removed and tested for TseF activity by including them, at a final dilution of 1:20 or 1:40, into the 3-d proliferation cultures containing LDH_B-primed Lyt-1^+2^- cells.

Trypsin Treatment of Cells. The Lyt-1^+2^- fraction of LDH_B-primed lymph-node cells was treated with trypsin (2.5 mg/ml) in the presence of DNAse I (20 μg/ml) according to the method described previously (26). The recovered cells were washed three times, cultured overnight in a medium containing 25% TCGF, and tested for functional activity in the 3-d proliferation assay, with or without previous exposure (6 h) to TseF.

Generation of Allorestricted T Cells. T cells recognizing antigen in the context of allogeneic A molecules were generated by in vitro priming to LDH_B on allogeneic APC, after the removal of alloreactive cells with 5-bromo-2′-deoxyuridine and light treatment. Here we used a recently described[2] modification of the original method (27).

Results

The Effect of Hybridoma-produced TseF on Th Cells is Antigen-specific and Mhc-Restricted. Hybridoma supernates, when included in the 3-d proliferation assay, inhibited to background levels the response of LDH_B-primed Lyt-1^+2^- cells from B10.AL and B10.A(2R) mice (unseparated cells from these strains are nonresponders to LDH_B because of the presence of Lyt-1^+2^+ Tse cells; references 15–17), but did not influence the proliferative response of KLH-primed and GAT-primed B10.A(2R) cells (Table I). Thus the suppressive effect of these factors is antigen-specific. However, the LDH_B-specific TseF completely suppressed the proliferative response of T cells to the

[2] Baxevanis, C. N., Z. A. Nagy, and J. Klein. 1983. The nature of the interaction between suppressor and helper T cells in the response to LDH_B. Manuscript submitted for publication.

TABLE II

Cross-Suppression of IgG_{2a}-Specific T-cell Proliferation by the LDH_B-Specific TseF

Source of supernatant*	Proliferative response of Th cells (Lyt-1^+2^-) from B10.A (4R) mice primed to UPC 10			
	Anti-UPC 10 response‡		Anti-LDH_B response‡	
	Δ cpm	S.I.	Δ cpm	S.I.
—	330117	117.2	1320	1.5
BW5147	308972	43.1	ND	
2RL-Ts-1	2287	1.3	ND	

* See footnotes to Table I.

‡ Proliferation of 4×10^5 T cells in the presence of 125 μg/ml of UPC 10 or 15 μg/ml of LDH_B.

ND, not done.

TABLE III

Time Course of Action of TseF on Th Cells

Preincubation of Th cells with TseF	Proliferative response of LDH_B-primed Th cells (Lyt-1^+2^-) after contact with 2RL-TsF1*				Proliferative response of GAT-primed Th cells (Lyt-1^+2^-) after contact with 2RL-TsF1*			
	Anti-LDH_B response‡		Con A response‡		Anti-GAT response‡		Con A response‡	
	Δ cpm	S.I.	Δ cpm	S.I.	Δ cpm	S.I.	Δ cpm	S.I.
0	78,851	(25.1)	126,195	(68.6)	15,748	(7.4)	138,986	(57.7)
30 min	80,476	(39.3)	113,528	(55.1)	17,067	(6.6)	107,438	(36.2)
1 h	92,667	(35.0)	114,597	(43.1)	15,366	(6.5)	110,571	(40.2)
2 h	76,531	(34.0)	110,592	(48.7)	18,171	(8.3)	103,527	(42.2)
4 h	125	(1.1)	105,389	(60.7)	15,003	(9.8)	102,832	(61.1)
8 h	−235	(0.9)	107,019	(70.6)	16,142	(8.8)	103,711	(51.2)

* Cells were incubated at 37°C with hybridoma supernate at a 1:2 dilution, washed, and tested for proliferation.

‡ The concentration of Con A was 25 μg/ml; LDH_B and GAT were used at concentrations given in Table I.

IgG_{2a} myeloma protein UPC 10 (Table II). This finding confirms our previous observation that the Tse cells involved in the anti-LDH_B and anti-IgG_{2a} responses cross-react completely (28). The crude hybridoma supernates caused complete suppression of T cell proliferation at a dilution up to more than 1:500 (data not shown). The data in Table III demonstrate that a short term (4 h) exposure of cells to the factor is sufficient to cause complete suppression, indicating that the factor is a TseF that acts directly on the Th cells. The interaction of TseF with Th is Mhc-restricted (Table IV). The strains used as Th donors map this restriction to the A_α-E_β interval, and excludes the involvement of all other *H-2* regions (also the *J* region), and of non-*H-2* genes. Since the action of the LDH_B-specific Tse cells on Th cells is also short-term, antigen-specific, and A-restricted, the hybridoma TseF can be considered as the secreted form of the molecule responsible for the Tse-Th cell interaction.

Mhc-Restricted Absorption of TseF Activity with LDH_B-Specific Th Cells. The suppres-

TABLE IV
H-2 Mapping of TseF Restriction

LDH$_B$-primed Th Cells (Lyt-1$^+$2$^-$)						Proliferative response of Th cells (Lyt-1$^+$2$^-$) treated with:*							
Strain	Alleles at *H-2* regions					BW5147		2RL-Ts-1		2RL-Ts-2		2RL-Ts-5	
	K	*A*	*J*	*E*	*D*	Δ cpm	(S.I.)	Δ cpm	(S.I.)	Δ cpm	(S.I.)	Δ cpm	(S.I.)
B10.A(2R)	*k*	*k*	*k*	*k*	*b*	155,412	(15.1)	−1	(1.0)	−2,163	(0.9)	1,471	(1.1)
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	130,368	(48.2)	−32	(1.0)	802	(1.2)	568	(1.1)
B10.A(5R)	*b*	*b*	*k*	*k*	*d*	123,249	(39.6)	115,453	(28.9)	127,165	(19.9)	110,223	(24.6)
B10.A(3R)	*b*	*b*	*b*	*k*	*d*	139,033	(12.5)	138,469	(12.6)	ND		137,574	(10.7)
B10.S(9R)	*s*	*s*	*k*	*k*	*d*	142,147	(23.4)	121,319	(19.7)	ND		116,946	(14.8)
B10.MBR	*b*	*k*	*k*	*k*	*q*	134,185	(61.4)	−91	(1.0)	421	(1.1)	381	(1.1)
B10.AQR	*q*	*k*	*k*	*k*	*d*	122,624	(17.7)	−718	(0.9)	ND		698	(1.1)
C57BL/6	*b*	*b*	*b*	*b*	*b*	131,065	(28.7)	133,404	(26.5)	146,938	(16.3)	156,367	(25.2)
CBA	*k*	*k*	*k*	*k*	*k*	303,645	(44.8)	2,535	(1.4)	2,809	(1.3)	1,494	(1.2)

* The TseF was added to cultures at a final dilution of 1:20.
ND, Not done.

TABLE V
Absorption of TseF with Antigen-Specific Th Cells

Source of supernatant	Absorbing cells*	Proliferative response of Th (Lyt-1$^+$2$^-$) cells to LDH$_B$		
		Strain	Δ cpm	S.I.
BW5147	None	B10.AL	59,792	29.2
2RL-Ts-5	None	B10.AL	329	1.1
2RL-Ts-5	LDH$_B$-Primed syngeneic Lyt-1$^+$2$^-$	B10.AL	57,581	22.1
2RL-Ts-5	GAT-Primed syngeneic Lyt-1$^+$2$^-$	B10.AL	103	1.0
BW5147	None	B10.TL	166,071	14.3
2RL-Ts-1	None	B10.TL	−59	1.0
2RL-Ts-1	LDH$_B$-Primed syngeneic Lyt-1$^+$2$^-$	B10.TL	150,383	12.5
2RL-Ts-1	LDH$_B$-Primed syngeneic Lyt-1$^+$2$^+$, Lyt-1$^-$2$^+$	B10.TL	511	1.0
BW5147	None	B10.AQR	115,704	17.7
2RL-Ts-5	None	B10.AQR	698	1.1
2RL-Ts-5	Unprimed B10.A(2R) spleen cells	B10.AQR	−962	0.9
2RL-Ts-5	LDH$_B$-Pulsed B10.A(2R) spleen cells	B10.AQR	74	1.0
2RL-Ts-5	Unprimed B10.A(4R) spleen cells	B10.AQR	767	1.1
2RL-Ts-5	LDH$_B$-Pulsed B10.A(4R) spleen cells	B10.AQR	205	1.0

* 1 ml of TseF diluted 1:4 or 1:10 was absorbed with 4×10^7 and 2×10^7 cells, respectively.

sive activity of hybridoma supernates can be removed completely by absorption with LDH$_B$-primed syngeneic [B10.A(2R)] Lyt-1$^+$2$^-$ cells (Table V). In contrast, syngeneic Lyt-2-bearing cells primed to LDH$_B$, or syngeneic Lyt-1$^+$2$^-$ cells primed to GAT do not absorb the factor. Similarly, LDH$_B$-pulsed or unpulsed spleen cells from unprimed syngeneic mice are unable to absorb the factor. Thus the TseF binds to the cells on which it acts (LDH$_B$-specific Th cells), but fails to bind to LDH$_B$-primed non-Th cells, to Th cells of irrelevant specificity, or to APC. The binding of TseF to Th cells is subject to the same genetic restriction as its functional activity, that is, the factor is only absorbed by LDH$_B$-primed Th cells that share genes in the A_α-E_β interval with

TABLE VI

A^k-Restricted Absorption of TseF with Th Cells

Source of TseF	Absorbing LDH_B-primed Lyt-1^+2^- cells*		Proliferative response of Th (Lyt-1^+2^-) cells		
	Strain	Alleles at *H-2* regions *K A J E D*	Strain	Δ cpm	S.I.
2RL-Ts-1	—	—	B10.A(2R)	−314	1.0
2RL-Ts-1	B10.A(2R)	*k k k k b*	B10.A(2R)	52,166	5.2
2RL-Ts-1	B10.A(4R)	*k k b b b*	B10.A(2R)	52,449	4.8
2RL-Ts-1	B10.A(5R)	*b b k k d*	B10.A(2R)	722	1.1
2RL-Ts-1	C57BL/6	*b b b b b*	B10.A(2R)	945	1.1
2RL-Ts-1	CBA	*k k k k k*	B10.A(2R)	51,589	4.5
2RL-Ts-5	—	—	B10.AQR	698	1.1
2RL-Ts-5	A.TFR1	*s k k k f*	B10.AQR	106,074	11.5
2RL-Ts-5	B10.S(9R)	*s s k k d*	B10.AQR	457	1.1

* 1 ml of 1:4 diluted TseF was absorbed with 2×10^7 cells.

the factor-producing cells (Table VI). Thus the factor acts after binding to its target cell in an antigen-specific and Mhc-restricted fashion.

Antigen Requirement of TseF–Th Cell Interaction. The antigen specificity of TseF-binding to Th cells can reflect either a direct receptor–receptor interaction (for example, idiotype–antiidiotype reaction) or an indirect interaction through an antigen bridge. To distinguish between these possibilities, we tested whether antigen is necessary for the TseF to act on Th cells. To conclusively demonstrate this point, one first has to ensure that the experimental system is devoid completely of antigen. This requirement is met in the case of the TseF used, since the factor is secreted by hybridomas in the absence of LDH_B. However, the Th cells might have carried LDH_B, and so, to preclude this possibility we trypsinized them to remove the surface-bound antigen. Because trypsin also attacks cell-membrane proteins (29), the cells were allowed to resynthesize their receptors during an overnight incubation in antigen-free medium. The interaction of these cells with TseF was then tested in a short term (6 h) culture, with or without LDH_B. The cells were then washed, and tested for antigen-specific proliferation. Suppression ensued during the 6-h exposure of Th cells to TseF only when antigen was added to the system (Table VII). Furthermore, trypsin-treated Th cells regenerated their capacity to proliferate to LDH_B but they were unable to absorb TseF in the absence of antigen (Z. Ikezawa, unpublished results). We conclude, therefore, that an antigen-bridge is necessary for the TseF to bind to and act on Th cells.

The Mechanism of Mhc-Restriction in the Interaction between TseF and Th Cells. The data in Tables IV and VI have demonstrated that the TseF only suppresses Th cells that share the $A_\alpha E_\beta$ interval genes with the factor-producing cells. We have previously shown that the same genetic restriction applies to the Tse–Th cell interaction in the anti-LDH_B response (17). Further analysis has revealed that the Tse–Th cell interaction is determined by the receptor of Th cells (anti-LDH_B + A^k) and the presence of A^k-alleles in the genome of Tse cells.[2] We inferred from these data that the interaction is based on the recognition by Th cells of A^k-controlled determinants expressed on the

TABLE VII

Antigen Requirement in the TseF–Th Cell Interaction: LDH_B-Primed B10.A(4R) Th ($Lyt\text{-}1^+2^-$) Cells after Trypsin Treatment and Overnight Incubation

Short-term (6 h) preincubation with		Proliferative response to LDH_B	
Supernatant*	LDH_B‡	Δ cpm	S.I.
BW5147 sup	−	158,776	25.1
BW5147 sup	+	146,072	24.0
2RL-TsF-1	−	162,980	24.1
2RL-TsF-1	+	2,293	1.3

* Cells were preincubated at 37°C with hybridoma supernates at a 1:4 dilution, washed and tested for proliferation.

‡ 15 μg/ml, when present.

TABLE VIII

Inhibition of TseF Activity by T Cell-specific Anti-A^k Antibodies

Pretreatment of factors with antibodies (final dilution)		Proliferative response of B10.A(4R) Th cells ($Lyt\text{-}1^+2^-$) after 6 h incubation with			
		BW5147 sup.*		2RL-TsF-5*	
		Δ cpm	(S.I.)	Δ cpm	(S.I.)
—	—	296,704	(89.1)	190	(1.0)
10.2.16:αIam.17	(1:200)	148,377	(54.2)	189	(1.1)
15.124.4:αIa.m2	(1:200)	189,470	(56.1)	−45	(1.0)
25.9.17:αA^b	(1:200)	237,411	(61.4)	684	(1.2)
13.4:αIa.m7	(1:1,000)	225,713	(64.0)	345	(1.1)
Y17:αIa.m44	(1:1,000)	218,754	(73.0)	646	(1.2)
1L9:αA^k-T	(1:800)	222,535	(75.5)	167,453	(53.6)
14P:αA^k-T	(1:800)	230,138	(69.9)	248,514	(68.4)

* Hybridoma supernates were used at a 1:10 final dilution.

Tse cells. If this hypothesis is valid, the TseF that exhibits identical genetic restriction to that of the Tse cell, must carry A^k-controlled antigenic determinants. We therefore tested whether A^k-specific monoclonal antibodies can abrogate the effect of TseF on Th cells. The factor was preincubated with the antibodies and then allowed to interact with LDH_B-primed $Lyt\text{-}1^+2^-$ cells for 6 h. The cells were then washed and tested for LDH_B-specific proliferation in a 3-d assay. As shown in Table VIII, antibodies against the known class II Mhc molecules (A and E) did not interfere with the activity of TseF. Interestingly, however, monoclonal antibodies IL9 and 14P that recognize A_α-E_β interval-controlled T-cell specific determinants (23) completely neutralized the TseF. Another antibody of the T-cell-specific "anti-A^k" series, 2L2, had the same effect, whereas antibody 1G8 that reacts with Tsi cells (T. Tada et al., unpublished data) had no effect on the TseF (Z. Ikezawa, unpublished results). Antibodies 1L9, 2L2, and 14P, when included in the assay cultures, neutralized the added TseF, but did not block the $A^k_\alpha A^k_\beta$-restricted proliferation of Th cells (Table IX). Furthermore, the antibodies did not affect the capacity of Th cells to absorb the TseF (Z. Ikezawa, unpublished data). Thus, the antibodies recognize A^k-like determinants carried by the factor, but absent from the APC and Th cells. We have investigated also whether

TABLE IX

T-Cell Specific Anti-A^k Antibodies Neutralize TseF But Do Not Block Th Cell Proliferation

Antibody added to cultures*	Proliferative response of B10.A(4R) Th ($Lyt\text{-}1^+2^-$) cells to LDH_B in the presence of:			
	BW5147 sup‡		2RL-TsF-1‡	
	Δ cpm	S.I.	Δ cpm	S.I.
None	329,473	34.0	657	1.1
1L9	227,318	20.1	138,255	13.3
2L2	217,104	17.7	115,385	10.4
14P	228,007	21.9	223,918	20.7

* Final dilution 1:800.
‡ Final dilution 1:40.

TABLE X

The Interaction of TseF With Th Cells Is Determined by the Anti-A^k Restriction Specificity of Th Cells

Th ($Lyt\text{-}1^+2^-$) cells		Supernatant added*	Monoclonal antibody added‡	Proliferative response to LDH_B§	
Strain	Receptor specificity			Δ cpm	S.I.
CBA	$LDH_B + A^k$	BW5147 sup	—	91,295	25.8
CBA	$LDH_B + A^k$	2RL-TsF-1	—	337	1.1
CBA	$LDH_B + A^k$	2RL-TsF-1	1L9(αA^k-T)	75,233	31.7
CBA	$LDH_B + A^k$	2RL-TsF-1	14P(αA^k-T)	81,538	33.9
CBA	$LDH_B + A^s$	BW5147 sup	—	82,398	38.3
CBA	$LDH_B + A^s$	2RL-TsF-1	—	94,446	49.0
B10.S	$LDH_B + A^k$	BW5147 sup	—	96,599	72.6
B10.S	$LDH_B + A^k$	2RL-TsF-1	—	−6	1.0
B10.S	$LDH_B + A^k$	2RL-TsF-1	1L9(αA^k-T)	55,593	31.7
B10.S	$LDH_B + A^k$	2RL-TsF-1	14P(αA^k-T)	75,607	44.4
B10.S	$LDH_B + A^s$	BW5147 sup	—	85,606	45.9
B10.S	$LDH_B + A^s$	2RL-TsF-1	—	84,024	43.7

* Final dilution 1:40.
‡ Final dilution 1:800.
§ Tested in the presence of the relevant APC (CBA and B10.S, respectively).

the interaction of A^k-like determinants of the TseF with Th cells depends on the Mhc haplotype or the receptor specificity of the Th cells. We generated in vitro CBA (*H-2^k*) and B10.S (*H-2^s*) Th cells that recognize LDH_B in a self-restricted manner (A^k- and A^s-restricted, respectively), or in the context of allogeneic A molecules (CBA anti-$LDH_B + A^s$, and B10.S anti-$LDH_B + A^k$). As shown in Table X, the Th cells, irrespective of their Mhc haplotype, are suppressed by the factor, provided that they recognize LDH_B together with A^k molecules. The interaction of A^k-like determinants of TseF with the anti-A^k receptors of Th cells is abrogated by the T-cell specific "anti-A^k" antibodies 1L9 and 14P. Thus, a functionally important part of the factor is an A^k-like moiety, which binds to the receptor site imposing A^k-restriction on the recognition of LDH_B by Th cells.

Discussion

In this communication we have functionally characterized a monoclonal TseF secreted constitutively by hybridomas that were made by fusing a long-term, LDH_B-specific Tse cell line with the BW5147 thymoma. In terms of specificity and function, the TseF is equivalent to the Tse cell itself (17), and can thus be considered the effector molecule of the latter. The factor consists of two functional parts, one that binds LDH_B, and another that carries or mimics the A^k determinant(s) recognized by the Th cells on the APC. For the suppression to occur, a double bond between TseF and Th cell has to be established, that is, the TseF must bind to the anti-LDH_B receptor of Th cells through an antigen bridge, and the A^k-like determinants of the factor must be bound by the anti-A^k receptor of the Th cell. (Whether the anti-LDH_B and anti-A^k binding sites of Th cells are physically linked remains unknown.) When one of these binding events is prevented from occurring by either the removal of LDH_B from the system, or blocking of the A^k-like moiety with antibodies, no suppression occurs. The crucial role of A^k-like determinants in suppression has also been demonstrated by an independent line of evidence, namely by the observation that LDH_B-primed T cells from suppressed nonresponder strains can be turned responder by including in the cultures any of the T-cell-specific monoclonal "anti-A^k" antibodies (23) used in this study (Z. Ikezawa et al., manuscript in preparation). The requirement for a dual recognition explains the antigen-specificity and the A^k-restriction of the interaction between the TseF (or the Tse cells) and the Th cells[2] (17). Once the TseF is bound to the Th cells, the suppression ensues within a matter of hours.

Many of the suppressor factors characterized so far resemble the TseF described here, in that they possess a binding site specific for either the immunizing antigen (4–7, 9–13, 30, 31) or a receptor-idiotype of the target cell (8), and carry Mhc-controlled antigenic determinants (4–10, 12, 30, 31). The anti-antigen- or antiidiotype-binding site determines the factor–target cell interaction in all known suppressor systems. The Mhc determinants, in many but not all systems, impose a genetic restriction on the factor–target cell interaction (4, 6–8, 12, 32). Thus, the interaction of these and some additional factors (33, 34) with their targets appears to involve dual recognition, similar to the LDH_B-specific TseF. One important difference between the LDH_B-specific TseF and all other known factors is that the former carries an $A_\alpha E_\beta$ interval-controlled moiety instead of determinants controlled by the so-called J region. This fortunate situation permitted us to analyze the A-like moiety by comparison with its well-characterized counterpart, the $A_\alpha A_\beta$ class II molecule. The data have demonstrated that there are determinants shared between APC-derived and TseF-derived A molecules, namely those that serve as restriction elements for antigen-recognition by Th cells. The factor-derived A-moiety carries additional determinants that are absent from APC (or B cell)-derived A molecules (those recognized by the T cell-specific monoclonal "anti-A^k" antibodies), and lacks serologically detectable determinants of the classical A molecule. Whether the two types of A molecule are controlled by different genes remains to be seen. Considering the relative paucity of class II genes in the $A_\alpha E_\beta$ interval (35), it is more likely that they represent different variants of a single gene product. By analogy, it is tempting to speculate that the so-called J molecule may be an analogous modification of another known class II gene product, perhaps the E_β chain.

Summary

Hybridomas secreting a monoclonal T suppressor-effector factor (TseF) were produced by fusion of a lactate dehydrogenase B (LDH_B)-specific long-term T suppressor-effector (Tse) cell line with the BW5147 thymoma. A short exposure (4 h) to TseF completely suppresses the antigen-specific and A-restricted proliferation of LDH_B-primed Lyt-1^+2^- [possibly helper (Th)] cells. The action of TseF on Th cells, as that of the Tse cells themselves, is antigen-specific and A-restricted. The interaction of TseF with Th cells involves two binding events, of which one occurs via antigen bridge, and the other represents the recognition of a factor-derived A^k-like moiety by the anti-A^k receptor of Th cells. The A^k-like moiety of the TseF carries the determinants that serve as restriction elements for antigen recognition by Th cells, and additional determinants demonstrable by T cell-specific monoclonal "anti-A^k" antibodies, however, it lacks serologically detectable determinants of the B cell-derived $A_\alpha A_\beta$ class II Mhc molecules.

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