

MONOCLONAL ALLOANTIBODIES SPECIFIC FOR THE CONSTANT REGION OF T CELL ANTIGEN RECEPTORS

By TAKESHI TOKUHISA, YOSHIKAZU KOMATSU, YOSHIAKI UCHIDA, AND
MASARU TANIGUCHI

From the Department of Immunology, School of Medicine, Chiba University, Chiba, Japan

The allotypic determinants exclusively expressed on suppressor T cells (Tsu^d)¹ or inducer of suppressor T cells (Tind^d) have been demonstrated (1, 2) by antisera or monoclonal antibodies that were made by immunizations of an immunoglobulin heavy chain (Igh) allotype congenic pair of mice (i.e., BALB/c anti-C.AL-20). The genes coding for the T cell allotypic determinants have unambiguously been mapped between the Igh constant region genes (*Igh-C*) and the prealbumin gene (*Pre-1*) on the 12th chromosome (3). Furthermore, the determinants seem to be present on the antigen receptor on suppressor T cells because the binding of idiotype-specific suppressor T cells to the idiotype-bearing Ig was blocked by the antiserum (1). Therefore, these results suggested that T cell antigen receptors can be encoded by genes linked to the *Igh* genes.

In fact, we have been able to demonstrate (4) that the antiserum made by immunizations of BALB/c (*H-2^d*, *Igh-1^a*) mice with T cell blasts from the Igh allotype congenic mice CB-20 (*H-2^d*, *Igh-1^b*) detects the allotypic determinants on the antigen-specific T cell factors, i.e., suppressor (TsF) or augmenting factor (TaF), which suppresses or augments the in vitro secondary IgG antibody response. Further evidence (4, 5) has suggested that the anti-CB-20 antiserum contains at least two distinct antibodies recognizing the constant region determinants on the antigen-binding molecules (C_t) of the functionally different T cell factors (i.e., TsF or TaF). Genetic analysis (5) of the determinants using the antiserum also indicated that the allotypic determinants on the T cell factors are encoded by genes linked to the *Igh-1^b* genes. These genes are distinctly located on the right side of the Igh variable-region (*Igh-V*) genes on the 12th chromosome because the anti-CB-20 column specifically reacted with T cell factors from BAB-14 mice that carry the *Igh-V* genes from *Igh-1^a* mice and the *Igh-C* genes from *Igh-1^b* mice.

Based on the above evidence, we tried to establish hybridoma-producing monoclonal antibodies recognizing the C_t determinants of the antigen-specific T cell factors (T cell-receptor molecules) expressed on the functionally distinct T cell subsets. Such monoclonal antibodies would be useful for characterizing the biochemical properties of the antigen-recognition units on T cell subsets. In this report, we describe the

¹ *Abbreviations used in this paper:* BPV, *Bordetella pertussis* vaccine; C, complement; Con A, concanavalin A; C_t, constant region of the antigen-binding chain of T cell factors; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; HAT, hypoxanthine, aminopterin, and thymidine; KLH, keyhole limpet hemocyanin; OVA, ovalbumin; PBS, phosphate-buffered saline; PEG, polyethylene glycol; P3U1, P3-X63Ag8-U1; PFC, plaque-forming cells; SRBC, sheep erythrocytes; T, thymus derived; TaF, augmenting T cell factor; TsF, suppressor T cell factor.

establishment of three characteristic monoclonal anti-CB-20 antibodies, each of which reacts with the distinct constant region determinants on the antigen-binding chain of TsF or TaF. By using these monoclonal antibodies, some functional and genetic properties of the T cell factors will also be described.

Materials and Methods

Animals. C57BL/6 CrSlc ($H-2^b$, $Igh-1^b$), BALB/c CrSlc ($H-2^d$, $Igh-1^a$) mice were purchased from Shizuoka Experimental Animal Laboratory, Co. Ltd., Hamamatsu, Japan. Igh allotype congenic mice, CB-20 ($H-2^d$, $Igh-1^b$), BAB-14 ($H-2^d$, $Igh-1^b$), C3H.SW/Hz (CSW; $H-2^b$, $Igh-1^j$), and CWB/Hz ($H-2^b$, $Igh-1^b$), have been maintained in our animal facility.

Antigens. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem Behring Corp., American Hoechst Corp., San Diego, CA. Ovalbumin (OVA), recrystallized five times, was obtained from Sigma Chemical Co., St Louis, MO. Dinitrophenylated KLH (DNP₇₇₀-KLH), and OVA (DNP₅-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. Anti-I-J^b, B10.A(5R) anti-B10.A(3R) was raised in our laboratory by the reciprocal immunization of the spleen and thymus cells. (A/J × B10.MBR)F₁ anti-B10.A(5R) (anti-I-A^bB^b) was kindly provided by Dr. D. H. Sachs, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Immunization. Immunization protocol to produce antibodies specific for T cell factors was described previously (4). Briefly, BALB/c mice were immunized intravenously with 10⁷ CB-20 spleen cells precultivated with 5 μg/ml of concanavalin A (Con A; Sigma Chemical Co.) for 48 h. The animals were immunized more than 10 times at weekly intervals.

Cell Fusion and Cloning. 3 d after the last immunization, cell hybridization was carried out by the method described elsewhere (6). In brief, 10⁸ primed spleen cells were mixed with 10⁷ P3-X63Ag8-U1 (P3U1) in a 50-ml tube and pelleted by centrifugation at 400 g. The cells were then resuspended in 1 ml prewarmed (37°C) 50% polyethylene glycol (PEG; 1,500 mol wt; Sigma Chemical Co.) by gentle shaking, followed by the dropwise addition of 1 ml of serum-free RPMI 1640 (GIBCO Laboratories, Grand Island Biochemical Co., Grand Island, NY). The cells were further diluted with RPMI 1640 and then centrifuged at 200 g for 5 min. The cell pellet was resuspended with warm medium containing 1 × 10⁻⁴ M hypoxanthine, 4 × 10⁻⁷ M aminopterin, and 1.6 × 10⁻⁵ M thymidine (HAT). 100 μl of the cell suspensions was distributed to each well of a 96-well tissue culture plate (3042; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA). Hybrids were cultured in HAT medium for 2–4 wk. Culture supernatants were added to the spleen cell culture to detect the antibody activity, as described below. Cells in the positive wells were cloned at least twice with limiting dilution.

Cytotoxic Test. The two-step cytotoxic assay was performed as described elsewhere (6). 10⁵ suppressor T cell hybridoma (34S-704) cells were incubated with 20 μl of diluted antiserum for 30 min at 4°C. After washing once, the cells were treated with 20 μl of rabbit complement (C) for 30 min at 37°C. 200 cells were counted per well under the light microscope by a trypan blue dye exclusion.

Quantitative Absorption of the Monoclonal Anti-CB-20 (7C5) with Spleen Cells. Quantitative absorption studies were carried out in a V-bottomed microtiter plate (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, VA). 30 μl of a 1:2,000 diluted culture supernatant from a hybridoma line (7C5) that showed the end point of maximum cytotoxicity was absorbed with graded numbers (3–192 × 10⁶ cells/ml × 30 μl) of spleen cells from various strains of mice for 1 h at 4°C. The supernatants were collected by centrifugation and then tested for the cytotoxic activities on the antigen-binding⁺, I-J⁺ suppressor T cell hybridoma cells (34S-704).

Preparation of the Antigen-specific Suppressor (TsF) and Augmenting T Cell Factor (TaF). The method used to obtain TsF and TaF was described in detail elsewhere (7). Briefly, mice were immunized intraperitoneally twice with 200 μg of KLH or OVA at a 2-wk interval. 2 wk after the last immunization, spleen cells were obtained and subsequently disrupted by freezing and thawing. The cell-free extracts were obtained by ultracentrifugation at 40,000 g for 1 h.

Absorption and Elution of TsF and TaF with Immunoabsorbent Columns. The cell-free extract

containing TsF and TaF was applied to immunoadsorbent columns composed of monoclonal antibodies and incubated for 1 h at 4°C. Effluent was collected by passing through the columns. After extensive washing with RPMI 1640 medium, the materials were then eluted from the columns with 2 ml of 0.175 M glycine-HCl buffer, pH 3.2, at 4°C. The eluates were instantly neutralized with 0.4 M sodium bicarbonate and dialyzed with phosphate-buffered saline (PBS), pH 7.2. They were then concentrated by vacuum pressure.

In Vitro Culture System. Modified Mishell-Dutton culture system using a 96-well microtiter plate (Falcon 3042) was used. 8×10^5 spleen cells from mice primed 6 wk previously with 100 μ g of DNP-KLH or DNP-OVA and 10^6 BPV were cultured in 0.2 ml of RPMI 1640 enriched with 10% fetal calf serum in the presence of 0.1 μ g/ml of DNP-KLH or DNP-OVA and 2×10^{-5} M 2-mercaptoethanol at 37°C in 5% CO₂ in air for 5 d. 10 μ l of cell-free extract equivalent to 2×10^6 spleen cells was added to the culture on day 0, to examine the TsF activity, or on day 2, for testing the TaF activity. 5 d later, numbers of anti-DNP IgG plaque-forming cells (PFC) were assayed by using DNP-coupled erythrocytes.

Statistical Analysis. For in vitro PFC assays, results were analyzed with a two-tailed Student's *t* test. Probability values of >10% were considered to be insignificant.

Results

Screening of the Monoclonal Anti-CB-20 Antibodies Specific for Functional T Cell Factors. Our previous studies (4) have shown that immunizations of BALB/c mice with Igh allotype-congenic spleen cells (CB-20) successfully produced antisera specific for the allotypic determinants of T cell factors (TsF or TaF). Therefore, BALB/c-derived myeloma cells (P3U1) were hybridized with spleen cells of BALB/c mice hyperimmunized with CB-20 spleen cells. To select hybridoma-producing antibodies specific for TsF or TaF, DNP-KLH-primed spleen cells were cultivated with hybridoma culture supernatant in the presence or absence of the KLH-specific T cell factors. As shown in Table I, culture supernatants of the 7C5, 7D1, or 6A4 clone did not show

TABLE I
Inhibitory Effects of Monoclonal BALB/c Anti-CB-20 Antibodies on the Activity of TsF or TaF

Monoclonal antibodies \ddagger added	IgG anti-DNP PFC/culture*				
	Experiment 1		Experiment 2		
	Materials added \S on day 0		Materials added \parallel on day 2		
	Medium	TsF	Medium	TaF	<i>P</i> value \P
Medium	3,100 \pm 620**	440 \pm 300	1,270 \pm 220	2,050 \pm 220	<0.001
7C5	2,820 \pm 550	2,300 \pm 260	ND $\ddagger\ddagger$	ND	—
7D1	2,940 \pm 700	2,490 \pm 390	ND	ND	—
6A4	ND	ND	1,030 \pm 200	1,230 \pm 260	NS $\S\S$

* 8×10^5 DNP-KLH-primed spleen cells from C57BL/6 mice were cultured in 0.2 ml of a 96-well microtiter plate in the presence of hybridoma supernatants and antigen-specific T cell factors.

\ddagger 50 μ l of hybridoma supernatant or medium was added to the in vitro secondary anti-DNP IgG response of spleen cells of C57BL/6 mice at the start of cultivation.

\S The cell-free extract equivalent to 2×10^6 spleen cells of KLH-primed C57BL/6 mice was added to the culture on day 0 to detect the TsF activity.

\parallel The cell-free extract equivalent to 2×10^6 spleen cells of KLH-primed C57BL/6 mice was added to the culture on day 2 to detect the TaF activity.

\P Significance level of difference from the spleen cell culture without TaF (control).

** Results are expressed as arithmetic mean numbers of anti-DNP IgG PFC of six cultures \pm SD.

$\ddagger\ddagger$ Not done.

$\S\S$ Not significant.

any effect on the in vitro secondary antibody response when they were cultured with spleen cells in the absence of the T cell factors. However, the hybridoma supernatants abrogated the KLH-TsF or KLH-TaF activity in the presence of T cell factors. These results suggest that the hybridoma antibodies specifically reacted with TsF or TaF and resulted in eliminating the activities of the T cell factors. The isotype of the 7C5, 7D1, or 6A4 antibody was found to be IgM, IgM, IgG₁, respectively, as determined by radioimmunoassay described elsewhere (8).

Monoclonal Anti-CB-20 (7C5, 6A4, and 7D1) Antibodies Recognizing Constant Region Determinants on Antigen-specific T Cell Factors. To examine the determinants of T cell factors recognized by monoclonal anti-CB-20 antibodies, the KLH- or OVA-specific T cell factors were absorbed with immunoadsorbent columns of the 7C5, 6A4, or 7D1 antibody. The materials were also eluted from the columns with 0.175 M glycine-HCl buffer, pH 3.2. The effluent or the eluate was added to the culture of DNP-KLH- or DNP-OVA-primed spleen cells on day 0, to detect the suppressor (TsF) activity, and also added on day 2, to determine the augmenting (TaF) activity. The data shown in Table II demonstrated that the 7C5 column absorbed the KLH-TsF and OVA-TsF but not TaF. On the contrary, the 6A4 reacted only with TaF. These results indicate that the 7C5 and 6A4 antibodies recognize the distinct constant region determinants exclusively expressed on the TsF and TaF, respectively. The 7D1 antibody was, however, shown to be directed to the constant region determinants shared in TsF and TaF because the 7D1 column absorbed both TsF and TaF.

Genetic Specificity of Monoclonal Anti-CB-20 (7C5, 6A4, and 7D1) Antibodies. Genetic specificities of the monoclonal anti-CB-20 antibodies were examined by absorption of KLH-TsF and KLH-TaF with immunoadsorbent columns composed of the 7C5, 6A4, or 7D1 antibody. The effluent or the eluate from these columns was added to the culture on day 0 and on day 2. As TsF or TaF has been demonstrated to suppress or enhance the in vitro secondary IgG responses of the *I-J-* or the *I-A*-compatible strains of mice (7, 9), respectively, the activities of KLH-TsF and KLH-TaF obtained from C57BL/6, C3H.SW, and CWB mice having the *I-J^b* and *I-A^b* haplotypes were tested in the responses of DNP-KLH-primed C57BL/6 (*H-2^b*) spleen cells. Similarly, KLH-TsF and KLH-TaF from *I-J^d* and *I-A^d* (BALB/c and BAB-14) mice were added to the cultures of DNP-KLH-primed BALB/c (*H-2^d*) spleen cells. Table III shows that the monoclonal antibody columns successfully absorbed the KLH-specific T cell factors from the *Igh-1^b* (C57BL/6, CWB, and BAB-14) mice but not those from BALB/c (*Igh-1^a*) and C3H.SW (*Igh-1^j*) mice. The results indicate that the 7C5, 6A4, and 7D1 antibodies recognize the allotypic determinants on TsF and TaF from the *Igh-1^b* mice.

The genetic specificity of the 7C5 antibody (IgM) specific for TsF was also investigated by a cytotoxic inhibition assay. The diluted culture supernatant of the 7C5 clone that showed an end point of maximum cytotoxic activity was absorbed with graded numbers of spleen cells from various strains of mice. The residual cytotoxic activity was tested on the suppressor T cell hybridoma. Fig. 1 shows the cytotoxic curves of the 7C5 antibody after absorption with spleen cells from C57BL/6 (*Igh-1^b*), BALB/c (*Igh-1^a*), BAB-14 (*Igh-1^b*), C3H.SW (*Igh-1^j*), and CWB (*Igh-1^b*) mice. The cytotoxic activity of the 7C5 was absorbed with spleen cells from *Igh-1^b* mice, such as C57BL/6, BAB-14, and CWB mice, but not those from BALB/c and C3H.SW mice with different Igh allotypes. It is, therefore, clear that the

TABLE II
Monoclonal BALB/c Anti-CB-20 Antibodies React with Constant Region Determinants of Antigen-specific T Cell Factors

C57BL/6 factor*		Materials added‡	IgG anti-DNP PFC/culture		
Specific for	Absorbed with		TsF§	TaF	P value¶
—	—	—	1,430 ± 230**	1,090 ± 160	—
KLH	—	Unabsorbed factor	160 ± 80	2,090 ± 270	<0.001
KLH	7C5	Effluent	1,500 ± 100	2,100 ± 270	<0.001
KLH	7C5	Eluate	180 ± 90	1,000 ± 370	NS‡‡
KLH	6A4	Effluent	220 ± 80	1,210 ± 130	NS
KLH	6A4	Eluate	1,440 ± 340	2,630 ± 260	<0.001
KLH	7D1	Effluent	1,420 ± 240	1,070 ± 140	NS
KLH	7D1	Eluate	230 ± 100	2,500 ± 460	<0.001
—	—	—	1,010 ± 130	1,610 ± 270	—
OVA	—	Unabsorbed factor	130 ± 50	3,330 ± 330	<0.001
OVA	7C5	Effluent	1,200 ± 160	3,070 ± 270	<0.001
OVA	7C5	Eluate	60 ± 20	1,480 ± 220	NS
OVA	6A4	Effluent	150 ± 100	1,280 ± 260	NS
OVA	6A4	Eluate	1,050 ± 200	2,900 ± 260	<0.001
OVA	7D1	Effluent	1,110 ± 200	1,610 ± 200	NS
OVA	7D1	Eluate	120 ± 100	3,720 ± 590	<0.001

* The cell-free extract equivalent to 2×10^8 spleen cells of KLH- or OVA-primed C57BL/6 mice was absorbed with and eluted from immunoadsorbent columns of monoclonal antibodies.

‡ The effluent or the eluate from the columns was added to the culture on day 0 (TsF) or on day 2 (TaF) to detect the TsF or TaF activity.

§ The KLH-specific or OVA-specific spleen cell extract from C57BL/6 mice was added on day 0 to the culture of DNP-KLH- (upper column) or DNP-OVA- (lower column) primed spleen cells from C57BL/6 mice, respectively.

|| The KLH-specific or OVA-specific spleen cell extract from C57BL/6 mice was added on day 2 to the culture of DNP-KLH- (upper column) or DNP-OVA- (lower column) primed spleen cells from C57BL/6 mice, respectively.

¶ Significance level of difference from the spleen cell culture without TaF (control).

** Results are expressed as arithmetic mean numbers of anti-DNP IgG PFC of six cultures \pm SD.

‡‡ Not significant.

7C5 antibody recognizes T cell surface products encoded by *Igh-1^b*-linked genes.

Allootypic Determinants Are Present on Antigen-binding Chains of T Cell Factors. It has been demonstrated (10, 11) that KLH-TsF is composed of two discrete molecules, i.e., the KLH-binding and the I-J-bearing chains in noncovalent association in the extracted TsF, and that these two chains, neither of which could exert any function by itself, reconstituted the TsF activity when mixed together. Therefore, the extracted KLH-TsF was absorbed with immunoadsorbent columns composed of KLH, 7C5, or anti-I-J^b to determine whether the 7C5 reacts with the KLH-binding or the I-J-encoded molecule. The effluents, or a mixture of these from any two of the columns, were added to the culture on day 0. The results shown in Table IV demonstrated that the TsF activity was completely absorbed with any of the columns. No suppressor activity was detected in the effluent from the columns. However, the mixture of the effluents from the KLH and anti-I-J^b or from the 7C5 and anti-I-J^b, but not those from the KLH and 7C5 columns, reconstituted the strong suppressor activities. From our previous experiments, together with the results shown in Table IV, the effluent

TABLE III
Genetic Specificity of Monoclonal BALB/c Anti-CB-20 Antibodies

Factor‡ absorbed with	Materials added	Added§ on day	IgG anti-DNP PFC/culture*				
			Factors derived from				
			C57BL/6	C3H.SW	CWB	BALB/c	BAB-14
—	—	—	1,610 ± 230	1,980 ± 240	4,220 ± 730	2,350 ± 80	1,720 ± 140
—	Unabsorbed factor	0	190 ± 90	180 ± 110	1,290 ± 240	140 ± 120	170 ± 150
7C5	Effluent	0	1,360 ± 210	100 ± 80	3,910 ± 610	240 ± 70	2,100 ± 130
7C5	Eluate	0	150 ± 60	1,970 ± 300	1,430 ± 480	2,260 ± 180	140 ± 50
7D1	Effluent	0	1,580 ± 110	100 ± 60	3,780 ± 120	230 ± 140	1,900 ± 220
7D1	Eluate	0	140 ± 60	1,920 ± 190	1,360 ± 200	2,360 ± 360	150 ± 80
—	—	—	1,370 ± 190	1,010 ± 180	1,140 ± 180	1,020 ± 70	1,750 ± 210
—	Unabsorbed factor	2	2,910 ± 390¶	2,260 ± 330¶	2,280 ± 310¶	2,480 ± 190¶	3,630 ± 620¶
6A4	Effluent	2	1,210 ± 130**	2,370 ± 160¶	1,125 ± 260**	2,730 ± 380¶	2,100 ± 360**
6A4	Eluate	2	2,630 ± 260¶	1,010 ± 160**	2,080 ± 180¶	1,110 ± 120**	3,740 ± 570¶
7D1	Effluent	2	1,260 ± 60**	2,180 ± 380¶	1,040 ± 50**	2,800 ± 430¶	1,730 ± 330**
7D1	Eluate	2	2,860 ± 360¶	1,120 ± 140**	2,170 ± 320¶	1,160 ± 170**	3,560 ± 100¶

* The KLH-TsF derived from C57BL/6 ($H-2^b, Igh-1^b$), C3H.SW ($H-2^b, Igh-1^j$), and CWB ($H-2^b, Igh-1^b$) mice was added to the culture of DNP-KLH-primed C57BL/6 mice. The KLH-TsF from BALB/c ($H-2^d, Igh-1^a$) and BAB-14 ($H-2^d, Igh-1^b$) mice was added to the culture of DNP-KLH-primed BALB/c mice.

‡ The cell-free extract equivalent to 2×10^6 spleen cells of KLH-primed mice was absorbed with the columns composed of monoclonal antibodies. See Table II.

§ See Table II.

|| Results are expressed as arithmetic mean numbers of anti-DNP IgG PFC of six cultures \pm SD.

¶ Significance level of difference from the spleen cell culture without TaF; $P < 0.001$.

** Significance level of difference from the spleen cell culture without TaF; $P > 0.1$ (NS).

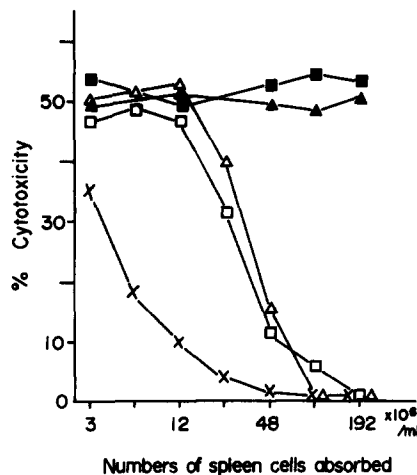


FIG. 1. Quantitative absorption analysis of the monoclonal BALB/c anti-CB-20 antibody (7C5) specific for TsF. 30 μ l of 1:2,000 diluted culture supernatant from the 7C5 clone was absorbed with graded numbers ($3-192 \times 10^6$ cells/ml \times 30 μ l) of the following spleen cells: C57BL/6 ($Igh-1^b$) (x), C3H.SW ($Igh-1^j$) (▲), CWB ($Igh-1^b$) (△), BALB/c ($Igh-1^a$) (■), and BAB-14 ($Igh-1^b$) (□). The residual cytotoxic activities were assayed on the KLH-specific suppressor T cell hybridoma (Materials and Methods). The background killing was $<10\%$.

from the anti-I-J^b column should contain the KLH-binding molecule. Similarly, the KLH column supplies the I-J molecules in the effluent. Because the 7C5 column behaves the same as the KLH column in Table IV, it is likely that 7C5 has the capacity to absorb the KLH-binding molecules but not the I-J molecules.

As TaF has been demonstrated to have the antigen-binding moiety and the I-A-

TABLE IV
Monoclonal 7C5 and 6A4 Antibodies Recognizing Antigen-binding Chains of Antigen-specific T Cell Factors

C57BL/6 factor absorbed with*						IgG anti-DNP PFC/culture‡		
None	KLH	Anti-I-J ^b	7C5	Anti-I-A ^b	6A4	Materials added on		
						Day 0	Day 2	P value§
—	—	—	—	—	—	1,740 ± 420	1,160 ± 160	—
2 × 10 ⁶ ¶	—	—	—	—	—	220 ± 170	2,170 ± 400	<0.001
—	4 × 10 ⁶	—	—	—	—	1,420 ± 130	1,180 ± 220	NS**
—	—	4 × 10 ⁶	—	—	—	1,700 ± 410	ND‡‡	
—	—	—	4 × 10 ⁶	—	—	1,630 ± 620	ND	
—	—	—	—	4 × 10 ⁶	—	ND	960 ± 90	>0.05
—	—	—	—	—	4 × 10 ⁶	ND	1,170 ± 260	NS
—	2 × 10 ⁶	2 × 10 ⁶	—	—	—	340 ± 180	ND	
—	2 × 10 ⁶	—	2 × 10 ⁶	—	—	1,380 ± 240	ND	
—	—	2 × 10 ⁶	2 × 10 ⁶	—	—	260 ± 110	ND	
—	2 × 10 ⁶	—	—	2 × 10 ⁶	—	ND	2,150 ± 330	<0.001
—	2 × 10 ⁶	—	—	—	2 × 10 ⁶	ND	1,170 ± 170	NS
—	—	—	—	2 × 10 ⁶	2 × 10 ⁶	ND	2,290 ± 200	<0.001

* The cell-free extract equivalent to 8 × 10⁶ spleen cells from KLH-primed C57BL/6 mice was adsorbed with immunoadsorbent columns.

‡ The effluent or the mixture of the one-half volume of effluents from these columns was added to the DNP-KLH-primed C57BL/6 spleen cell culture on day 0 or on day 2.

§ Significance level of difference from the spleen cell culture without TaF (control).

|| Results are expressed as mean numbers of anti-DNP IgG PFC of six cultures ± SD.

¶ Cell numbers equivalent to the amount of absorbed factors added to the culture.

** Not significant.

‡‡ Not done.

subregion gene products, the experiment was designed to examine whether TaF is composed of two chains like TsF and whether the determinant defined by 6A4 is present on the antigen-binding molecule. For this, a similar experiment described in the previous section using the anti-I-A^b and 6A4 columns was carried out. In this experiment, the extracted T cell factor was added to the culture on day 2. The results in Table IV show that the augmenting activity was reconstituted by the mixture of the effluents from the KLH and anti-I-A^b or from the 6A4 and anti-I-A^b columns, neither of which showed any TaF activity by itself. The results indicate that TaF is also composed of two chains and that the 6A4 antibody reacted the molecules with antigen-binding moiety of TaF. It is, therefore, concluded that 7C5 and 6A4 recognize the determinants expressed on the antigen-binding molecules of the functional subsets of T cells.

Discussion

The hybridomas producing antibodies specific for allotypic markers on the antigen-binding molecules of functional T cell factors have been established by the fusion of BALB/c myeloma (P3U1) and BALB/c spleen cells hyperimmunized with Con A-stimulated spleen cells of Igh allotype congenic mice (CB-20). The antibodies were successfully selected by functional assays as shown in Table I. The 7C5 and 7D1 antibodies (IgM) were seen to have the ability to block the TsF activity when they

were added together with TsF into the in vitro secondary antibody responses. Similarly, the addition of the 6A4 antibody (IgG₁) to the culture resulted in abrogating the TaF activity.

The reactivity of monoclonal antibodies was further investigated. It is demonstrated in Table III that the antibodies absorb suppressor or augmenting T cell factors from *Igh-1^b* mice (i.e., C57BL/6, BAB-14, and CWB) but not those from mice with different Igh allotypes, such as BALB/c (*Igh-1^b*) and C3H.SW (*Igh-1^j*) mice. Therefore, the monoclonal antibodies seem to recognize the allotypic determinants on antigen-specific T cell factors from *Igh-1^b* mice. Furthermore, radioimmunoassay using ¹²⁵I-labeled 7C5, 6A4, and 7D1 antibodies excluded the possibility that they might react with allotypic determinants of the *Igh-1^b* Ig or C57BL/6 splenic B cells (data not shown). Thus, the determinants appear to be different from the allotype markers of Ig and B cells. Genetic specificities of the 7C5 and 7D1 antibodies were also confirmed by quantitative absorption with spleen cells from various strains of mice. It can, therefore, be strongly suggested that the monoclonal alloantibodies described here are specific for the allotype markers on T cells and their factors encoded by genes linked to the *Igh-1^b* gene cluster. Fig. 1 also shows that the capacity of C57BL/6 spleen cells to absorb the 7C5 antibody activity was about five times more efficient than that of the spleen cells from other *Igh-1^b* mice. No explanation for this can be offered at this time. It is, however, possible that this might be because of the difference in the number of 7C5 positive cells among the various mouse strains.

The antigen-specific suppressor T cell factor has been demonstrated to bear the antigen-binding structure as well as the constant region determinants that were defined by the xenoantiserum against mouse suppressor T cell factors (rabbit anti-mouse TsF) (12). No genetic analysis of constant determinants on TsF was performed. However, recent studies reported by Owen et al. (1) and Tokuhisa and Taniguchi (4) have strongly suggested that T cell allotypic determinants encoded by genes linked to the *Igh* locus are expressed on T cell antigen receptors or antigen-specific T cell factors. In this respect, the monoclonal antibodies (7C5, 7D1, and 6A4) were found to recognize constant region determinants on antigen-binding molecules of T cell factors (Tables II and IV) because the monoclonal antibody has the ability to absorb both KLH- and OVA-specific T cell factors, both of which should have the distinct variable region structure. However, our results in Tables II and IV could not exclude the possibility that the antibody might react with variable region determinants in case there might be allotypic markers in the variable region of the T cell receptor similar to the allotypes of the rabbit Ig (13).

It is clear that the monoclonal 6A4 and 7C5 antibodies recognize the distinct antigen-binding molecule on different functional T cell factors (Tables II and IV). However, the 7D1 antibody recognizes the shared constant determinants between TsF and TaF (Table II). Therefore, at least two discrete genes coding for the C_t of T cell receptors can be supposed to be present on the 12th chromosome. Genetic specificities of the monoclonal antibodies have further shown that both genes coding for the C_t molecules locate to the right side of the *Igh-V* genes on the 12th chromosome because the antibodies absorb T cell factors from *Igh-1^b* mice and BAB-14 mice carrying the *Igh-C* genes from *Igh-1^b* mice and the *Igh-V* genes from *Igh-1^a* mice (14). In this respect, the existence of a gene complex, downstream from *Igh-1* and coding for a family of T cell isotypes (Tsu^d, Tind^d, or Tthy^d), has been proposed by Owen et al.

(15). We cannot determine at this time whether or not the C_t determinants defined by our monoclonal reagents are identical to Tsu^d and $Tind^d$. In any event, it is possible that the cluster of genes coding for the constant region determinants of antigen-receptors on functionally different T cell subsets locates adjacent to the *Igh-C* genes on the 12th chromosome.

We previously reported the existence of the antigen-specific T cell factor (TaF) that augments antibody responses (7). This factor was shown to possess an I region determinant encoded by a unique gene in the *I-A* subregion that is not identical to that coding for the B cell Ia antigen (7, 16). The results in Table IV demonstrate that TaF is composed of two distinct chains, one possessing the antigen-binding moiety and the other the product of a gene in the *I-A* subregion and that the 6A4 antibody reacts with the molecules having the KLH-binding moiety. Therefore, the antigen-specific TaF has a structural resemblance to the antigen-specific TsF (10). TaF was also found to have similar characteristics to TsF in that the mixture of the two chains, neither of which could exert any functional activity by itself, could successfully reconstitute the augmenting activity.

From our previous studies on the hybridoma-derived TsF (10, 11), together with the data presented here, it is strongly suggested that I region gene products being associated with the antigen-binding chain of T cell factors act as elements to convey T cell functions (i.e., suppressing or augmenting). As antigen-binding chains have the C_t determinants unique to functionally different T cell subsets, it is also likely that the C_t molecules serve as functional domains of T cell factors similarly to the Fc domain of Ig. In this regard, it is imperative to conduct investigations of the biological roles of the molecules responsible for the expression of the functions of the T cell factors. Further studies using monoclonal antibodies (7C5 and 6A4), currently in progress, are awaiting results to answer these questions.

Summary

We established three distinct monoclonal antibodies (7C5, 7D1; IgM and 6A4; IgG₁) by the fusion of P3U1 and BALB/c (*Igh-1^a*) spleen cells hyperimmunized with T cell blasts from the immunoglobulin heavy chain (Igh) allotype congenic CB-20 (*Igh-1^b*) mice. The 7C5 or 6A4 antibody reacts with the constant region determinants on the antigen-binding molecule (C_t) of the antigen-specific suppressor T cell factor (TsF) or augmenting T cell factor (TaF), respectively. The 7D1 antibody, however, recognizes the shared determinants on the C_t molecules of TsF and TaF. Genetic studies of determinants recognized by these monoclonal antibodies have also suggested that the distinct C_t molecules of TsF and TaF are encoded by two discrete genes linked to the *Igh-1^b* genes, which are located on the right side of the variable genes of Igh on the 12th chromosome. By using the immunoadsorbent columns of 6A4 antibody and anti-I-A^b, TaF, in a manner similar to TsF, was demonstrated to be composed of two chains, i.e., the C_t molecules and the I-A-encoded products. Furthermore, the C_t -bearing molecules were shown to possess the antigen-binding moiety.

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References

1. Owen, F. L., A. Finnergar, E. R. Gates, and P. D. Gottlieb. 1979. A mature T lymphocyte subpopulation marker closely linked to the Igh-1 allotype CH locus. *Eur. J. Immunol.* **9**:948.
2. Spurrll, G. M., and F. L. Owen. 1981. A family of T-cell alloantigen linked to Igh-1. *Nature (Lond.)*. **293**:742.
3. Owen, F. L., R. Riblet, and B. A. Taylor. 1981. The suppressor cell alloantigen Tsu^d maps near immunoglobulin allotype genes and may be a heavy chain constant-region marker on a T cell receptor. *J. Exp. Med.* **153**:801.
4. Tokuhisa, T., and M. Taniguchi. 1982. Two distinct allotypic determinants on the antigen-specific suppressor and enhancing T cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. *J. Exp. Med.* **155**:126.
5. Tokuhisa, T., and M. Taniguchi. 1982. Constant region determinants on the antigen-binding chain of the suppressor T cell factor. *Nature (Lond.)*. In press.
6. Kanno, M., S. Kobayashi, T. Tokuhisa, I. Takei, N. Shinohara, and M. Taniguchi. 1981. Monoclonal antibodies that recognize the product controlled by a gene in the I-J subregion of the mouse H-2 complex. *J. Exp. Med.* **154**:1290.
7. Tokuhisa, T., M. Taniguchi, K. Ohumura, and T. Tada. 1978. An antigen-specific I region gene product that augments the antibody response. *J. Immunol.* **120**:414.
8. Herzenberg, L. A., S. J. Black, T. Tokuhisa, and L. A. Herzenberg. 1980. Memory B cells at successive stages of differentiation: affinity maturation and the role of IgD receptor. *J. Exp. Med.* **151**:1071.
9. Taniguchi, M., T. Tada, and T. Tokuhisa. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. III. Dual gene control of the T-cell mediated suppression of the antibody response. *J. Exp. Med.* **144**:20.
10. Taniguchi, M., I. Takei, and T. Tada. 1980. Functional and molecular organization of an antigen-specific suppressor factor derived from a T cell hybridoma. *Nature (Lond.)*. **283**:227.
11. Taniguchi, M., T. Saito, I. Takei, and T. Tokuhisa. 1981. Presence of interchain disulfide bonds between two gene products that compose the secreted form of an antigen-specific suppressor factor. *J. Exp. Med.* **153**:1672.
12. Kontiainen, S., and M. Feldmann. 1979. Structural characteristics of antigen-specific suppressor factors: definition of "constant" region and "variable" region determinants. *Thymus*. **1**:59.
13. Mage, R. G. 1975. Allotype suppression in rabbits: effects of anti-allotype antisera upon expression of immunoglobulin genes. *Transplant. Rev.* **27**:84.
14. Riblet, R., B. Blomberg, M. Weigert, R. Liberman, B. A. Taylor, and M. Potter. 1975. Genetics of mouse antibodies. I. Linkage of the dextran response locus, V_H-DEX, to allotype. *Eur. J. Immunol.* **5**:775.
15. Owen, F. L., G. M. Spurrll, and E. Panageas. 1982. Tthy^d, a new thymocyte alloantigen linked to Igh-1. Implications for a switch mechanism for T cell antigen receptors. *J. Exp. Med.* **155**:52.
16. Hiramatsu, K., A. Ochi, S. Miyatani, A. Segawa, and T. Tada. 1982. Monoclonal antibodies against unique I-region gene products expressed only on mature functional T cells. *Nature (Lond.)*. **296**:666.