SOLUBLE FACTORS IN TOLERANCE AND CONTACT SENSITIVITY TO 2,4-DINITROFLUOROBENZENE IN MICE

III. Histocompatibility Antigens Associated with the Hapten Dinitrophenol Serve as Target Molecules on 2,4-Dinitrofluorobenzene-immune T Cells for Soluble Suppressor Factor*

By JOHN W. MOORHEAD‡

From the Division of Clinical Immunology, Departments of Medicine and of Microbiology and Immunology, University of Colorado Medical School, Denver, Colorado 80262

There are now numerous examples of suppressor molecules released by mouse T cells. Both specific and nonspecific factors, regulating humoral or cell-mediated responses, have been identified and partially characterized (1-15). In some cases, the factors express determinants encoded by genes in the I region of the major histocompatibility complex (MHC)[1] (3), specifically the I-J (7-9, 11), or I-C region (15). The precise role of these Ia determinants in the factor-mediated suppression is not clear. In at least two instances, however, it appears that they interact with acceptor molecules encoded by the homologous I region on the target cell(s) (16-19), thus imparting a genetic restriction on the action of the suppressor molecules. The nature of this interaction, i.e., like-like or lock-and-key, has not been established.

Previous papers in this series have described a soluble suppressor factor (SSF) which suppresses contact sensitivity (CS) to 2,4-dinitrofluorobenzene (DNFB) (20, 21). This factor is released in vitro by cultures of suppressor T cell-containing lymph node (LN) cells from DNFB-tolerant mice. SSF is antigen (hapten) specific, and recent experiments show that the factor carries determinants encoded by the I region (I-C) of the MHC (J. Moorhead and C. S. David. Unpublished observations). Suppression by SSF is genetically restricted (21). However, in contrast to other factors which carry I-region determinants and require I-region homology with the target cells to suppress, SSF-mediated suppression requires that the donors of DNFB-immune LN cells and of SSF share either the H-2K and/or the H-2D region of the MHC. Thus, acceptor or target molecules for SSF appear to be coded for by genes within the H-2K and H-2D loci. The purpose of the studies reported here was to investigate the nature of these acceptor sites. The results indicate that the acceptor molecules for SSF are expressed

* Supported by United States Public Health Service grant AI-12993.

‡ Recipient of National Institutes of Health Research Career Development award K04-AI-00125.

[1] *Abbreviations used in this paper:* CS, contact sensitivity; Cy, cyclophosphamide; DNFB, 2,4-dinitrofluorobenzene; DNP, dinitrophenol; FCA, Freund's complete adjuvant; LN, lymph node; MHC, major histocompatibility complex; RAMIg, rabbit anti-mouse Ig; RGG, rabbit gammaglobulin; SSF, soluble suppressor factor; TNP, trinitrophenol; Ts-aux, auxillary T-suppressor cells.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/79/12/1432/16 $1.00
Volume 150 December 1979 1432-1447

only on DNFB-immune T cells and consist of histocompatibility antigens associated with the hapten dinitrophenol (DNP). The origin and role of these acceptor molecules is discussed.

Materials and Methods

Animals. CBA, C57B1/10, A/J, $C_3D_2F_1$, B10.BR, and B10.D2 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were obtained from the AMC Cancer Research Center, Lakewood, Colo. A.TH, A.TL, and B10.A(4R) mice were bred in our animal care facilities.

Sensitization with DNFB or 2,4,2-trinitrochlorobenzene (TNCB). To serve as donors of DNFB-immune LN cells, mice were sensitized with DNFB (Eastman Kodak Co., Rochester, N. Y.) by two daily paintings of 25 μl of 0.5% DNFB on the clipped abdomen and 5 μl on the footpads and ears. TNCB (Matheson, Coleman, and Bell, Cincinnati, Ohio) sensitivity was induced by a single painting of 100 μl of 7% TNCB on the clipped abdomen and 5 μl on the footpads and ears. DNFB-immune LN cells were taken 3 d after the last painting, whereas TNCB-immune LN cells were taken 4 d after the single painting.

Transfer of contact sensitivity. 3 d after sensitization with DNFB, single cell suspensions of draining LN were prepared and 4×10^7 to 5×10^7 cells injected i.v. into normal syngeneic recipients. The recipients and negative controls were challenged within 1 h after cell transfer by applying 20 μl of 0.2% DNFB on the dorsal side of each ear. Increased ear swelling (δ) was measured 24 h later with an engineer's micrometer and expressed in units of 10^{-4} in.

Antisera. Anti-H-2^d and anti-H-2^k were produced in C57B1/10 mice immunized with spleen and LN cells from B10.D2 or B10.BR mice, respectively. Anti-Ia^k was produced in A.TH mice by immunizing with A.TL spleen cells. The specificity and cytoxicity of this serum has been previously described (22). Anti-H-$2D^d$ ([B10.AKM × A.SW]F_1 anti-A.TH) and anti-H-$2K^k$ (A.TL anti-A.AL) were kindly provided by Dr. Chella David. Anti-DNP was produced in BALB/c mice by three immunizations with DNP coupled to rabbit gammaglobulin (DNP_{30}-RGG) emulsified in Freund's complete adjuvant (FCA). Anti-trinitrophenol (TNP) was produced in rabbits by three immunizations with TNP_{32}-RGG emulsified in FCA. Anti-brain-associated-θ (anti-BA-θ) was produced in rabbits according to Golub (23). Before use, the anti-DNP and anti-TNP sera were extensively adsorbed with mouse spleen, thymus, and LN cells and the anti-BA-θ serum was adsorbed with mouse bone marrow and erythrocytes. The anti-H-2 sera were tested for specificity and cytotoxicity using a microassay described by Pincus and Gordon (24).

Purification of anti-DNP antibodies. 2 ml of BALB/c anti-DNP serum was slowly passed over 5 ml of packed Sepharose beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) conjugated with DNP_{24}-KLH and the column then washed extensively with phosphate-buffered saline (PBS, pH 7.2). The bound antibodies were eluted with 0.1 M NH_4OH, the eluate neutralized immediately with 1 N HCl, dialyzed against PBS and concentrated by filtration through an Amicon XM100 membrane (Amicon Corp., Scientific Sys. Div., Lexington, Mass.). To insure that functional antibody was recovered, the eluate was tested by primary binding using ^{3}H-DNP-L-lysine (5×10^{-8} M DNP) and by Ouchterlony gel diffusion using a variety of DNP conjugates.

Preparation of Suppressor and Control Supernates. Suppressor and control supernates were prepared as described in detail previously (20). Briefly, mice were injected i.v. on day 0 with DNBS (750 mg/kg) and on day 5, they were painted on the clipped abdomen with DNFB. The next day, peripheral and mesenteric LN cells were placed in culture (10^7 cells/ml) in RPMI-1640 medium supplemented with serum and antibiotics. After 48 h, cell-free supernates were collected and stored at −20°C. Control supernates were prepared from mice not injected with 2,4-dinitrobenzene sulfonate (DNBS) but which were painted with DNFB.

Purification of DNFB-immune LN T and B cells. Purified T cells were prepared by passing immune LN cells through a nylon wool column as previously described (22) according to the method of Julius et al. (25). Nonadherent cells were collected and monitored for B cells and macrophages by immunofluorescent staining using fluorescein-labeled polyvalent rabbit anti-mouse Ig (RAMIg) and esterase staining, respectively. In three replicate experiments, the purified T cells contained 0–2% B cells and <1% macrophages.

Populations enriched in B cells were prepared by treating immune LN cells with anti-BA-θ serum (10^8 cells/ml of 1:10 diluted serum, 4°C for 30 min) plus guinea pig complement (10^8 cells/ml of 1:6 serum, 37°C for 30 min). Dead cells were removed by Ficoll/Isopaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.) gradient centrifugation (26). Interface cells were collected, viable cells determined by trypan blue dye exclusion, and B cells enumerated by immunofluorescent staining with RAMIg. In two replicate experiments, >96% of the cells were viable of which >80% were Ig^+.

Preparation of Peritoneal Exudate Cells. Peritoneal exudate cells were induced by intraperitoneal injection of 3 ml of thioglycollate medium. The cells were harvested by lavage 4 d later. The cells were 98% macrophages, as judged by esterase staining.

Preparation of Adsorbing LN Cells. Because large numbers of LN cells were needed for adsorption studies, T-cell-enriched, rather than purified populations, were prepared. This was done by suspending normal or immune LN cells in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) medium supplemented with 5% fetal calf serum and antibiotics and incubating the cells for 90 min at 37°C on plastic tissue culture dishes (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif). Nonadherent cells were recovered by gentle washing with warm medium. Cell recovery ranged from 65 to 80%. Immunofluorescent staining of the cells showed a depletion of Ig^+ cells from 25 to 30% in the starting population to 10% or less. Esterase-positive cells decreased from 12 to 15 to 5% or less. Thus, as a result of depletion of both Ig^+ cells and adherent macrophages, a cell population which was enriched in T cells was obtained.

Adsorption of Supernates. 5 ml of suppressor supernate was incubated with 2×10^8 LN cells or 10^8 macrophages for 1 h at 37°C with intermittent mixing. In some experiments, the cells were pretreated with antiserum (see Results) for 1 h at 4°C and then washed before being used for adsorption. The supernates were cleared by centrifugation and tested for suppressor activity on freshly prepared DNFB-immune LN cells.

Treatment of Immune LN Cells with Supernate. DNFB-immune LN cells were incubated with 5 ml of control, suppressor or adsorbed suppressor supernatant (5×10^7 cells/ml) for 1 h at 37°C. The cells were then washed twice and $4\text{–}5 \times 10^7$ cells injected i.v. into normal syngeneic recipients. Ear challenge and measurement of ear swelling were done as previously described. The suppressor effect of SSF was determined by its ability to suppress the expression of CS by the transferred LN cells. The suppressor activity of the adsorbed supernate was then compared to the suppressor activity of the unadsorbed supernate and the results expressed as the percent suppressor activity removed by adsorption.

Results

Suppression of Purified DNFB-immune T Cells by SSF. Initial experiments were done to determine if T cells could interact with and be suppressed directly by SSF. To accomplish this, LN cells were prepared from DNFB-immune BALB/c mice and a portion of the cells were purified on nylon wool columns. Unseparated and purified T cells were then incubated with BALB/c-derived control or suppressor supernate, washed, and transferred to normal syngeneic recipients. In this and all other experiments, the suppressor effect of SSF was determined by its ability to suppress the expression of CS by LN cells from DNFB-immune mice that were transferred into normal syngeneic recipients. Results of two experiments are shown in Table I. Both unseparated LN cells and purified T cells transferred CS. Both cell populations were suppressed equally by SSF. These results indicate that DNFB-immune T cells express the acceptor molecules for SSF and interact directly with the factor.

Cyclophosphamide (Cy) Pretreatment of Donors of DNFB-immune LN Cells Does Not Alter Their Sensitivity to SSF-mediated Suppression. Recent studies in our laboratory have identified a subset of T cells in DNFB-immune mice which are required for suppression of the efferent limb of contact sensitivity by suppressor T cells (27). This cell population is found only in DNFB-immune mice, it is IJ^+ and its precursors are

TABLE I
*Suppression of Purified DNFB-Immune T Cells by SSF**

Supernate	DNFB-immune LN cells transferred	Exp. 1		Exp. 2	
		Δ Ear swelling‡	Suppression	Δ Ear swelling	Suppression
		$\times 10^{-4}$ *in* ± *SEM*	%	$\times 10^{-4}$ *in* ± *SEM*	%
Control	Unseparated	34.4 ± 2.3		39.4 ± 3.3	
Suppressor	Unseparated	1.7 ± 0.6	95	8.3 ± 1.9	79
Control	Purified T cells	31.6 ± 3.1		34.1 ± 2.7	
Suppressor	Purified T cells	1.8 ± 1.3	94	6.0 ± 1.1	82

* Unseparated LN cells (24% Ig^+ in Exp. 1 and 19% Ig^+ in Exp. 2) and nylon wool purified LN T cells (0% Ig^+ in Exp. 1 and 2% Ig^+ in Exp. 2) from DNFB-immune BALB/c mice were treated in vitro with BALB/c-derived control or suppressor supernate and transferred to normal syngeneic recipients. Increased ear swelling was measured 24 h later as an index of transfer of contact sensitivity.

‡ Ear swelling in recipients of immune cells minus ear swelling in negative controls (ear challenge only).

sensitive to Cy. We have called these cells auxillary T-suppressor cells (Ts-aux). Ts-aux is distinct from the effector T-cell population (T_{DH}) which is Ia^- (22) and whose precursors are insensitive to Cy (27, 28). Because Ts-aux has an obligatory role in suppression by one type of suppressor T cell, experiments were done to determine if it was also required for suppression by SSF. BALB/c mice were injected i.v. with 200 mg/kg Cy, a dose which removes all Ts-aux activity (27). Control mice received saline. 2 d later, all of the mice were sensitized with DNFB. Immune LN cells were prepared 4 d later, treated in vitro with BALB/c control or suppressor supernate and transferred to normal syngeneic recipients. These recipients plus normal controls were then ear challenged and the ear swelling was measured 24 h later. Results of two experiments are given in Table II. Cy treatment of donors of immune LN cells had no effect on their sensitivity to suppression by SSF. Thus, we conclude that the Ts-aux population is not required for suppression of DNFB-immune T cells by SSF.

Ability of Various Cell Populations to Adsorb SSF. The results in Table I indicate that DNFB-immune-purified T cells can interact with SSF. To determine if other cells are able to interact with the factor, the adsorbing capacity of various cell populations was tested. BALB/c-derived suppressor supernates were adsorbed with whole LN cells, T-cell-depleted LN cells or macrophages from normal or immune syngeneic donors. The adsorbed supernates were then tested for suppressor activity on freshly prepared DNFB-immune BALB/c LN cells. The results are given in Table III. Of the five cell populations tested only DNFB-immune BALB/c LN cells adsorbed suppressor activity. LN cells or macrophages from normal BALB/c mice or TNCB-immune BALB/c LN had no absorbing activity. More important, depletion of T cells from DNFB-immune LN cells eliminated the ability of the remaining cells to adsorb the factor. Similar results were obtained when CBA-derived suppressor supernates were adsorbed with various populations of cells from normal or immune CBA mice, i.e., only DNFB-immune, T-cell-enriched LN cells adsorbed the suppressor factor (results not shown). Collectively, these results indicate that the only cells able to interact with SSF are DNFB-immune T cells.

Genetic Restriction of Suppressor Factor Adsorption. As described previously, SSF-mediated suppression of DNFB sensitivity requires that the donor of immune LN cells and of SSF share either the H-2K and/or H-2D region of the MHC (21). Thus, to begin to investigate the nature of the acceptor sites for SSF, suppressor supernates

TABLE II

*DNFB-Immune LN Cells from Cyclophosphamide Pretreated Donors are Suppressed by SSF**

Supernate	Pretreatment of donors of immune LN cells	Exp. 1		Exp. 2	
		Δ Ear Swelling‡	Suppression	Δ Ear Swelling	Suppression
		× 10^{-4} *in* ± *SEM*	%	× 10^{-4} *in* ± *SEM*	%
Control	Saline	27.5 ± 2.6		29.4 ± 3.6	
Suppressor	Saline	9.1 ± 1.7	67	8.2 ± 2.3	72
Control	Cyclophosphamide	24.9 ± 2.9		34.2 ± 3.7	
Suppressor	Cyclophosphamide	5.4 ± 0.6	78	7.0 ± 1.7	80

* BALB/c mice were injected i.v. on day −2 with 200 mg/kg cyclophosphamide. Control mice received saline. On days 0 and 1, all the mice were sensitized with DNFB and immune LN cells were harvested on day 4. These cells were then treated with BALB/c control or suppressor supernate and transferred to normal syngeneic recipients. Increased ear swelling was measured 24 h later as an index of transfer of contact sensitivity.

‡ See footnote in Table I.

TABLE III

Ability of Various Cell Populations to Adsorb Suppressor Factor

BALB/c supernate	Cells used to adsorb supernate	Δ Ear swelling‡	Suppressor activity removed by adsorption
		× 10^{-4} *in* ± *SEM*	%
Control	None	38.8 ± 3.7	
Suppressor	None	7.0 ± 1.3	
Suppressor	DNFB-immune BALB/c LN	36.7 ± 2.4	93.5
Suppressor	T-cell-depleted DNFB-immune BALB/c LN	6.5 ± 2.0	0
Suppressor	TNCB-immune BALB/c LN	8.2 ± 2.1	6.4
Suppressor	Normal BALB/c LN	7.7 ± 1.8	3.6
Suppressor	Normal BALB/c macrophages	7.5 ± 2.3	3.2

* 5 ml of suppressor supernate was incubated with 2 × 10^8 LN cells or 10^8 macrophages for 1 h at 37°C. The supernates were then cleared by centrifugation and tested for suppressor activity on freshly prepared syngeneic DNFB-immune LN cells.

‡ See footnote in Table I.

were adsorbed with DNFB-immune LN cells from different H-2 haplotype mice. As before, the adsorbed supernates were tested for suppressor activity on freshly prepared DNFB-immune LN cells from appropriate strains. All experiments have been done at least two times and representative results are given in Table IV.

In Exp. 1, BALB/c suppressor supernate was adsorbed with syngeneic or allogeneic (CBA) immune LN cells and the supernatants tested on immune BALB/c LN cells. The suppressor factor was adsorbed only by the syngeneic cells. The reciprocal experiment with respect to H-2K and H-2D is shown in Exp. 2. In this case, CBA-derived SSF (which was tested on CBA-immune LN cells) was completely adsorbed by B10.BR-immune LN cells (MHC identical with CBA) but no adsorption occurred with A.TL-immune LN cells (the I region was identical with CBA). The results indicate that homology at H-2K and/or H-2D is required for factor adsorption. To explore this point in more detail, experiments were done in which suppressor supernates were adsorbed with immune LN cells from mice sharing either the H-2D or H-2K region of the MHC with the factor-producing strain. In Exp. 3, A/J (H-$2K^k$, H-$2D^d$)-derived supernate was adsorbed with four different immune LN populations

TABLE IV

Genetic Requirements of Suppressor Factor Adsorption

Exp.	Supernate	DNFB-immune LN cells used to adsorb supernate*	Δ Ear swelling‡	Suppression activity removed by adsorption
			$\times 10^{-4}$ *in* ± *SEM*	%
1	BALB/c control	None	31.6 ± 3.7	
	BALB/c suppressor	None	3.7 ± 1.9	
	BALB/c suppressor	BALB/c	31.1 ± 3.0	98.2
	BALB/c suppressor	CBA	5.2 ± 1.3	5.6
2	CBA control	None	28.5 ± 2.2	
	CBA suppressor	None	4.9 ± 0.6	
	CBA suppressor	B10.BR	26.6 ± 3.3	92.0
	CBA suppressor	A.TL	5.1 ± 1.6	1.5
3	A/J control	None	30.6 ± 2.5	
	A/J suppressor	None	6.0 ± 1.9	
	A/J suppressor	A/J	28.0 ± 2.2	89.5
	A/J suppressor	BALB/c	26.3 ± 3.9	82.6
	A/J suppressor	A.TH	26.9 ± 3.3	85.0
	A/J suppressor	C57BL/10	7.1 ± 0.9	4.8
4	A/J control	None	35.2 ± 2.7	
	A/J suppressor	None	8.6 ± 1.4	
	A/J suppressor	A/J	33.7 ± 3.3	94.3
	A/J suppressor	CBA	32.6 ± 3.9	90.2
	A/J suppressor	B10.A(4R)	32.0 ± 3.7	88.0
	A/J suppressor	C57BL/10	10.2 ± 1.7	6.3
5	$C_3D_2F_1$ control	None	42.3 ± 5.0	
	$C_3D_2F_1$ suppressor	None	9.8 ± 1.5	
	$C_3D_2F_1$ suppressor	$C_3D_2F_1$	41.4 ± 2.9	97.2
	$C_3D_2F_1$ suppressor	BALB/c	38.9 ± 3.9	90.4
	$C_3D_2F_1$ suppressor	CBA	12.2 ± 2.3	7.5
6	$C_3D_2F_1$ control	None	39.3 ± 4.1	
	$C_3D_2F_1$ suppressor	None	7.7 ± 2.6	
	$C_3D_2F_1$ suppressor	$C_3D_2F_1$	35.7 ± 3.7	88.1
	$C_3D_2F_1$ suppressor	CBA	37.2 ± 4.1	93.4
	$C_3D_2F_1$ suppressor	BALB/c	9.1 ± 0.9	5.5

* 2×10^8 DNFB-immune LN cells were used to adsorb 5 ml of suppressor supernate for 1 h at 37°C. The supernates were cleared by centrifugation and tested for suppressor activity on freshly prepared DNFB-immune LN cells.

‡ See footnote in Table I.

and then tested on DNFB-immune BALB/c LN cells (H-$2K^d$, H-$2D^d$). Suppressor factor was adsorbed equally well by immune LN cells from A/J (syngeneic), BALB/c (IC→H-2D identical), and most important, A.TH (H-2D identical) mice. Completely allogeneic cells (C57Bl/10) did not adsorb the factor. Similar results are shown in Exp. 4 with respect to the H-2K locus. Here, A/J (H-$2K^k$, H-$2D^d$) suppressor factor was adsorbed and then tested on DNFB-immune CBA LN cells (H-$2K^k$, H-$2D^k$). Factor adsorption occurred with immune LN cells from A/J (syngeneic), CBA

(H-2K→IE identical), and B10.A(4R) (H-2K, IA identical) mice but not with LN cells from allogeneic C57B1/10 mice. Although B10.A(4R) and CBA mice share both the H-2K and portions of the I region with A/J, adsorption of the factor is most likely occurring via the H-2K homology because A.TL LN cells which are syngeneic in the I region with CBA do not adsorb CBA factor (see Exp. 2). Thus, the data indicate that identity at either H-2K or H-2D is both necessary and sufficient to adsorb the suppressor factor.

Rich et al. have previously shown that F_1-derived MLR suppressor supernates contain two distinct populations of suppressor factors, one specific for each parental haplotype (29). To explore this possibility in the contact sensitivity system, suppressor supernate derived from $C_3D_2F_1$ mice (H-2^k × H-2^d) was adsorbed with immune LN cells from BALB/c (H-2^d) or CBA (H-2^k) mice and then tested for suppressor activity. In Exp. 5, the F_1 supernates were tested on DNFB-immune BALB/c LN cells. Adsorption with BALB/c LN cells removed the suppressor activity whereas adsorption with CBA LN cells had no effect. The reciprocol is shown in Exp. 6. Here the supernates were tested on DNFB-immune CBA LN cells. Factor adsorption occurred with CBA LN cells but not with BALB/c LN cells. Thus, the data indicate that the F_1 supernates contain at least two suppressor factors, each specific for one parental haplotype and each specifically adsorbed by immune LN cells of that relevant H-2 haplotype.

Adsorption of Suppressor Factor is Blocked by Treating Immune LN Cells with Antibodies against H-2K or H-2D Determinants. If H-2K and H-2D gene products on DNFB-immune T cells serve as target molecules for SSF, then blocking these determinants with antibodies should inhibit adsorption of the factor. To evaluate this, DNFB-immune LN cells were treated for 1 h at 4°C with different anti-H-2 reagents (10^8 cells/ml in 1:10 diluted serum), washed, and then used to adsorb suppressor supernates. The supernates were then tested for suppressor activity on DNFB-immune LN cells from appropriate strains. In all experiments, the antiserum-treated cells were monitored for antibody-binding by immunofluorescent staining with RAMIg. Results of these experiments are given in Table V.

As shown in Exp. 1, pretreatment of immune BALB/c LN cells with anti-H-2^d (B10 anti-B10.D2; 99% of the cells were found positive by IF staining) completely blocked adsorption of the factor. Treating the cells with NMS had no effect (9% positive by IF staining). This experiment was repeated using immune CBA LN cells and CBA-derived suppressor factor (Exp. 2). Treating the immune cells with anti-H-2^k serum (B10 anti-B10.BR; 96% of the cells were found positive by IF staining) blocked factor adsorption. In contrast, treating the cells with anti-Ia^k serum (A.TH anti-A.TL; 13% of the cells positive by IF staining compared with 7% in NMS-treated cells) had no effect. This suggests that the blocking by anti-H-2 serum is a result of antibodies directed against gene products of the H-2K and H-2D loci, i.e., histocompatibility antigens. To verify this, experiments were done using antiserum specific for H-$2D^d$ or H-$2K^k$ determinants. In Exp. 3, DNFB-immune LN cells from BALB/c mice (H-$2D^d$) were treated with NMS or anti-H-$2D^d$ serum (10% of NMS treated and 100% of antiserum-treated cells were positive by IF staining). The washed cells were then used to adsorb A/J suppressor factor which was then tested on freshly prepared BALB/c DNFB-immune LN cells. As shown, treating the cells with anti-H-$2D^d$ completely blocked their ability to adsorb the factor. In Exp. 4, H-$2K^k$ determinants

TABLE V

Adsorption of Suppressor Factor by Immune LN Cells is Blocked by Pretreating the Cells with Antibodies Against H-2K or H-2D Determinants

Exp.	Supernate	DNFB-immune LN cells used to adsorb supernate	Pretreatment of immune LN cells before adsorption*	Δ Ear swelling‡	Suppressor activity removed by adsorption
				$\times 10^{-4}$ *in* ± *SEM*	%
1	BALB/c control	None	None	44.2 ± 5.1	
	BALB/c suppressor	None	None	7.3 ± 1.7	
	BALB/c suppressor	BALB/c	NMS	40.2 ± 3.3	89.4
	BALB/c suppressor	BALB/c	Anti-H-2^d	8.2 ± 2.0	2.3
2	CBA control	None	None	35.1 ± 3.7	
	CBA suppressor	None	None	5.1 ± 1.7	
	CBA suppressor	CBA	NMS	32.6 ± 4.1	91.2
	CBA suppressor	CBA	Anti-H-2^k	8.0 ± 1.6	8.1
	CBA suppressor	CBA	Anti-Ia^k	33.3 ± 2.9	94.0
3	A/J control	None	None	33.4 ± 3.6	
	A/J suppressor	None	None	7.0 ± 1.6	
	A/J suppressor	BALB/c	NMS	28.7 ± 3.0	82.1
	A/J suppressor	BALB/c	Anti-H-$2D^d$	6.6 ± 1.1	0
4	A/J control	None	None	26.7 ± 1.9	
	A/J suppressor	None	None	2.4 ± 0.6	
	A/J suppressor	CBA	NMS	26.4 ± 2.2	98.8
	A/J suppressor	CBA	Anti-H-$2K^k$	2.2 ± 1.0	0
5	BALB/c control	None	None	41.9 ± 3.8	
	BALB/c suppressor	None	None	8.0 ± 1.1	
	BALB/c suppressor	$C_3D_2F_1$	NMS	37.0 ± 2.8	85.6
	BALB/c suppressor	$C_3D_2F_1$	Anti-H-2^d	7.4 ± 1.7	0
	BALB/c suppressor	$C_3D_2F_1$	Anti-H-2^k	35.6 ± 3.6	81.5
6	CBA control	None	None	32.3 ± 3.3	
	CBA suppressor	None	None	4.2 ± 0.7	
	CBA suppressor	$C_3D_2F_1$	NMS	29.5 ± 2.2	90.0
	CBA suppressor	$C_3D_2F_1$	Anti-H-2^k	6.4 ± 0.4	7.8
	CBA suppressor	$C_3D_2F_1$	Anti-H-2^d	27.7 ± 3.0	83.6

* DNFB-immune LN cells were treated for 1 h at 4°C with NMS or anti-H-2 reagents. After washing, 2×10^8 cells were used to adsorb 5-ml alliquots of suppressor supernate for 1 h at 37°C. The supernates were cleared by centrifugation and tested for suppressor activity on freshly prepared DNFB-immune LN cells.

‡ See footnote in Table I.

were investigated. Immune LN cells from CBA mice (H-$2K^k$) were treated with NMS or anti-H-$2K^k$ (6% of NMS-treated and 99% of antiserum-treated cells positive by IF staining) and then used to adsorb A/J-derived suppressor factor. The supernates were tested on DNFB-immune CBA LN cells. Treating the immune CBA LN cells with anti-H-$2K^k$ completely blocked factor adsorption by the cells. These results provide direct evidence that H-2K and H-2D determinants on DNFB-immune T cells serve as target molecules for SSF.

To verify the specificity of the serum blocking in a more critical way, additional experiments were done in which F_1 DNFB-immune LN cells were treated with anti-H-2 sera specific for each parental haplotype. The washed cells were then used to adsorb suppressor supernates which were specific for one of the two haplotypes. If specific blocking occurs, then F_1 LN cells treated with anti-H-2 serum against P_1 haplotype should not adsorb SSF directed against that haplotype. However, F_1 cells treated with anti-H-2 serum against the P_2 haplotype should adsorb the factor and vice versa. Results are shown in Exps. 5 and 6. In Exp. 5, $C_3D_2F_1$ immune LN cells (H-2^k × H-2^d) were treated with either NMS, anti-H-2^d (B10 anti-B10.D2), or anti-H-2^k (B10 anti-B10.BR) serum (7% of NMS-treated, 99% of anti-H-2^d-treated, and 100% of anti-H-2^k-treated cells were determined positive by IF staining). The cells were then used to adsorb BALB/c-derived suppressor factor which suppresses DNFB-immune T cells expressing H-$2K^d$ or H-$2D^d$ determinants. The adsorbed supernates were then tested for suppression using DNFB-immune BALB/c LN cells. F_1 LN cells treated with anti-H-2^d were unable to adsorb the BALB/c suppressor factor. However, the F_1 cells treated with either NMS or anti-H-2^k serum (the irrelevant serum with respect to the specificity of the factor) adsorbed suppressor activity equally well. Results of the reciprocol experiment are shown in Exp. 6. In this case, CBA suppressor supernates which suppress DNFB-immune T cells expressing H-$2K^k$ or H-$2D^k$ determinants were adsorbed and tested on immune CBA LN cells. As shown, F_1 LN cells treated with anti-H-2^k (97% positive by IF staining) were unable to adsorb CBA-derived suppressor factor. On the other hand, F_1 cells treated with anti-H-2^d serum (98% positive by IF staining) which in Exp. 5 were unable to adsorb the BALB/c suppressor factor, adsorbed the CBA suppressor factor equally as well as did NMS-treated cells (8% positive by IF staining).

Adsorption of Suppressor Factor is Blocked by Treating Immune LN Cells with Anti-DNP. SSF has affinity and specificity for the hapten DNP (20). Thus, the possibility was considered that the antigen specificity of SSF could be a result of, in part, the presence of DNP on the immune T cell membrane, associated in some way with histocompatibility antigens. If so, then treating DNFB-immune LN cells with anti-DNP antibodies should block their ability to adsorb the suppressor factor. To investigate this, DNFB-immune BALB/c LN cells were treated with either NMS (10^8 cells/ml in 1:10 dilution) or affinity purified mouse anti-DNP antibodies (10^8 cells/ml, 25 μg anti-DNP/ml) for 1 h at 4°C. The cells were then washed and used to adsorb BALB/c-derived suppersson factor which was then tested on DNFB-immune BALB/c LN cells. The results are shown in Table VI. As shown in Exp. 1, treating the immune LN cells with anti-DNP antibodies completely blocked the adsorption of the suppressor factor. Similar experiments were done using whole anti-TNP serum prepared in rabbits (Exp. 2). Treating with anti-TNP serum also blocked the adsorbing capacity of the immune LN cells. To control for possible nonspecific steric inhibition, immune LN cells were pretreated with rabbit anti-BA-θ serum. This serum, although binding to >80% of the LN cells as measured by IF staining with fluorescein-labeled goat anti-rabbit Ig, did not block the adsorption of SSF.

Discussion

Previous studies on SSF-mediated suppression of contact sensitivity have indicated that the acceptor or target molecules on DNFB-immune LN cells for the suppressor

TABLE VI

Adsorption of Suppressor Factor by Immune LN Cells is Blocked by Pretreating the Cells with Antibodies Against DNP or TNP

Exp.	Supernate	DNFB-immune LN cells used to adsorb supernate	Pretreatment of LN cells before adsorption*	Δ Ear swelling‡	Suppression activity removed by adsorption
				$\times 10^{-4}$ *in* ± *SEM*	%
1	BALB/c control	None	None	37.4 ± 3.9	
	BALB/c suppressor	None	None	9.0 ± 1.6	
	BALB/c suppressor	BALB/c	NMS	34.8 ± 2.6	90.8
	BALB/c suppressor	BALB/c	Anti-DNP	9.4 ± 1.2	1.5
2	BALB/c control	None	None	43.6 ± 4.1	
	BALB/c suppressor	None	None	10.2 ± 1.7	
	BALB/c suppressor	BALB/c	NRS	37.5 ± 2.9	81.8
	BALB/c suppressor	BALB/c	Anti-TNP	12.0 ± 0.6	5.8
	BALB/c suppressor	BALB/c	Anti-BA-θ	41.4 ± 3.3	93.4

* DNFB-immune BALB/c LN cells were incubated for 1 h at 4°C with either NMS or anti-DNP (Exp. 1) or NRS, anti-TNP or anti-BA-θ (Exp. 2). After washing, 2×10^8 cells from each cell suspension were used to adsorb 5-ml alliquots of suppressor supernate for 1 h at 37°C. The supernates were cleared by centrifugation and tested for suppressor activity on freshly prepared syngeneic DNFB-immune LN cells.

‡ See footnote in Table I.

factor are coded for by genes in the H-2K and/or H-2D regions of the MHC (21). In the present study, adsorption experiments were done to investigate the nature of these acceptor molecules and to determine the cell type(s) on which they are expressed. The experimental results establish the following points: (*a*) Purified LN T cells from DNFB-immune mice express acceptor molecules for SSF; (*b*) pretreating mice with Cy before sensitization with DNFB does not alter the sensitivity of immune LN cells from these mice to suppression by SSF; (*c*) acceptor molecules for SSF are expressed only on DNFB-immune T cells. Normal T cells, macrophages, T cell-depleted DNFB-immune LN cells (B cells), or TNCB-immune LN cells do not absorb the suppressor factor and thus, do not express the target molecules; (*d*) for DNFB-immune T cells to adsorb the factor, they must share either the H-2K or H-2D region of the MHC with the factor-producing strain; and (*e*) factor adsorption can be blocked by treating the immune T cells with antibodies specific for H-2K or H-2D determinants or for the hapten DNP.

The finding that purified populations of immune LN cells are suppressed by SSF indicates that T cells can interact directly with the factor. This conclusion is supported by the experiments which showed that immune T cells are the only cell type capable of adsorbing the suppressor activity. Furthermore, pretreatment of mice with Cy at doses known to eliminate precursors of suppressor cells (28) and suppressor amplifier (30) or suppressor auxillary cells (27) does not alter the sensitivity of DNFB-immune T cells from these animals to suppression by SSF. Thus, it appears that suppression is mediated by interaction of the factor with immune LN T cells. We believe this interaction occurs directly with the effector T-cell population and does not require the participation of other T-cell subsets.

These results differ from previous reports by Zembala and Asherson (2) and more recently by Ptak et al. (31) concerning their studies on the suppressor factor for TNCB

contact sensitivity. These investigators have shown that this factor is completely adsorbed by normal peritoneal macrophages which are then able to suppress TNCB-immune T cells. Both living and heat-killed macrophages are able to adsorb the factor and adsorption is inhibited by blocking the macrophage Fc receptor with aggregated IgG. In addition, syngeneic, allogeneic and xenogeneic macrophages adsorb the factor equally well. My results show that macrophages do not adsorb the suppressor factor specific for DNFB contact sensitivity (Table III). Cell numbers as high as 50×10^6 macrophages/ml have been used with no detectable loss of suppressor activity. Furthermore, macrophages which have been used to adsorb the supernates are not suppressive when cotransferred with DNFB-immune LN cells to normal recipients (not shown).

An explanation for these different findings is not readily apparent. However, it should be noted that although the DNFB and TNCB factors are produced in essentially the same way, significant differences between them are known to exist. For example, SSF cannot be adsorbed by anti-DNP or -TNP affinity columns (20) whereas the TNCB factor is adsorbed by, and can be recovered from, the same columns (our unpublished results and personal communication from Mark Greene and Robert Cone). The TNCB factor has been shown to carry determinants encoded by genes in the I-J region of the MHC (11); i.e., it is specifically adsorbed by affinity columns conjugated with anti-IJ antibodies. We have confirmed this finding but have been unable to adsorb SSF on the same columns. Rather, SSF is adsorbed by columns conjugated with antibodies specific for I-C region determinants (J. W. Moorhead and C. S. David. Unpublished observations.). Finally, suppression by SSF is genetically restricted (21). No restrictions for suppression by the TNCB factor have been reported. Thus, the dichotomy with respect to adsorption by macrophages may be yet another difference between the factors and their mode of action. However, at the present time, we cannot rule out a possible role for macrophages in SSF-mediated suppression. It is possible that after SSF is bound to T cells, certain conformational changes occur in the molecule such that macrophages can not bind the factor. This would be similar to changes which are known to occur in the Fc portion of IgG antibodies following binding to antigen. Such an interaction may have an essential role in the ultimate suppression of the immune T cells. Experiments to explore this possibility are currently in progress.

For enriched populations of DNFB-immune T cells to adsorb SSF, identity at the H-2K and/or H-2D region of the MHC is required between the donor of the immune T cells and the donor of the factor. These restrictions coincide precisely with the genetic requirements for suppression (21). The essential role of histocompatibility determinants was further shown in the blocking experiments. Antibodies specific for H-2K or H-2D antigens completely blocked the ability of immune LN T cells to adsorb the factor, whereas antibodies against Ia or θ-antigens had no effect. This blocking of adsorption is highly specific. F_1 DNFB-immune LN cells, treated with anti-H-2 serum specific for the P_1 haplotype, were unable to adsorb SSF specific for that haplotype. However, F_1 cells treated with anti-H-2 serum specific for the P_2 haplotype, adsorbed the P_1 suppressor factor as well as F_1 cells treated with NMS. Similar results were obtained with suppressor factor specific for the P_2 haplotype, i.e., F_1 LN cells treated with anti-H-2 specific for P_2 haplotype did not adsorb the P_2 factor, whereas F_1 cells treated with anti-H-2 specific for P_1 were not inhibited. These

results provide strong evidence that histocompatibility antigens play an integral and essential role in the binding of SSF.

Nevertheless, histocompatibility antigens alone do not serve as the target molecules for SSF. This was shown in two ways. First, syngeneic populations of normal LN cells, T cell-depleted DNFB-immune LN cells and TNCB-immune LN cells, all of which express the relevant histocompatibility antigens, do not adsorb the suppressor factor. Second and more important, treatment of T-cell-enriched populations of DNFB-immune LN cells with anti-DNP or -TNP antibodies blocks their ability to adsorb the suppressor factor. Because these antisera were extensively adsorbed with normal lymphocytes before use and the antibodies for one serum were purified on a DNP-affinity column, their ability to block factor adsorption indicates that the immune T cells have DNP on their surface. Together with the blocking by anti-H-2 antibodies, these results indicate that the target molecules on DNFB-immune T cells for SSF are comprised of histocompatibility antigens associated with the hapten DNP.

These data are quite similar to previous reports by Schmitt-Verhulst et al. (32) and Burakoff et al. (33) concerning cytolytic T cells induced in vitro against hapten-modified autologous lymphocytes. These authors have shown that lysis by the T cells is inhibited by pretreating the target cells with antibodies directed against either the histocompatibility antigens or the hapten. Thus, it appears that these cytolytic T cells and SSF recognize similar determinants on the membranes of their respective target cells. This similarity might indicate that SSF actually represents soluble cytolytic T cell receptors shed in vitro by the cultured LN cells. This seems unlikely, however, because recent experiments in our laboratories indicate that cytolytic T cells are neither present in mice, nor can they be induced in cultures of LN cells from mice, treated in ways similar to those used to induce SSF (R. Cleveland. Unpublished results.).

The ability of either anti-H-2 or anti-DNP antibodies alone to block adsorption indicates that the factor must bind or recognize both moieties simultaneously. We do not know, however, whether the factor is monovalent and recognizes DNP and H-2 as a single determinant, i.e., like altered-self, or divalent and recognizes the two as independent determinants.

Several experimental findings must be considered in analyzing this question. We have previously shown that SSF is adsorbed by DNP-KLH affinity columns (20). Thus, the factor has affinity for DNP alone which suggests that it has a receptor or receptors specific for DNP. However, DNFB-immune LN cells allogeneic with the factor-producing strain are not suppressed by (20, 21), and do not adsorb, the factor even though they carry DNP on their surface, i.e., the T cells have the right hapten but the wrong H-2 antigens. This would seem to favor single determinant recognition although lack of adsorption in this case may also involve hapten density (see below). We have attempted to resolve this by adsorbing supernates with normal LN lymphocytes coupled in vitro with DNP (34). These cells contain ~2–3 μg of DNP/10^7 cells and we have used 2.5×10^7 cells to adsorb 5 ml of supernate. Thus far, we have found that DNP-lymphocytes have an adsorption profile exactly like DNFB-immune LN cells. That is, DNP-syngeneic lymphocytes (syngeneic with the factor-producing strain) completely adsorb the factor whereas DNP-allogeneic lymphocytes do not adsorb the factor (J. W. Moorhead. Unpublished observations.). These findings indicate that adsorption by DNP-syngeneic lymphocytes, like adsorption by DNFB-

immune T cells, involves more than factor recognition of hapten alone.

To reconcile these apparent contradictory findings, I have formulated the following working hypothesis with respect to the factor and its binding requirements: the factor is a multivalent molecule with at least two receptor sites specific for DNP and at least one receptor site specific for H-2. For the factor to form a stable bond with its respective target molecules, at least two receptor sites must be occupied. In the case of syngeneic DNFB-immune T cells or syngeneic DNP-labeled LN cells, this requirement is satisfied by the factor binding to DNP and H-2 determinants. For DNP-KLH affinity columns, binding occurs via two receptors for DNP. This is possible because of the high density of DNP molecules on the protein carrier. The factor does not bind to allogeneic DNFB-immune T cells or allogeneic DNP-labeled LN cells because simultaneous binding of the two receptors does not occur. The H-2-specific receptor does not bind because the wrong H-2 is expressed by the cells. Two receptors for DNP are not occupied because that hapten density on the cell surface is not sufficient to allow the necessary cross-linking.

An interesting question raised by the blocking studies in this report has to do with the origin and function of the hapten DNP which is present on the surface of the immune T cells. It is not generally thought that immune T cells carry membrane-bound antigen. There are at least two possible explanations for this finding. First, as a result of the highly reactive nature of the antigen, DNFB, it is possible that the hapten is bound directly to the cell surface. The association with histocompatibility antigens would then occur as a random event. All LN cells, rather than just T cells, should then have hapten bound to their membrane and should be able to adsorb the factor. The experiments in which T-cell-depleted LN cells from DNFB-immune mice failed to adsorb the suppressor molecules indicate that this is not the case.

An alternative explanation that we think best explains the data is that a complex of hapten plus histocompatibility determinants, shed by other cells, is passively bound to the membrane of the immune T cells. We believe that during the process of immunization with DNFB, antigen-presenting cells (macrophages?) shed small amounts of soluble membrane material, i.e., DNP complexed with H-2 determinants. Immune T cells then bind this material via some of their antigen receptors. The bound complexes serve as targets for the suppressor factor thus rendering the T cells sensitive to this form of immunoregulation. Because the complex serves only to focus the suppressor factor on the T cells, immune cells which do not have the complex on their surface would be insensitive to SSF-mediated suppression but should still be able to transfer immunity. Recent unpublished results in my laboratory support this model. DNFB-immune LN cells, after overnight culture in vitro, are able to transfer contact sensitivity at levels comparable to freshly prepared LN cells. However, the cultured LN cells are not suppressed by SSF nor are they able to adsorb the suppressor activity from the supernates.

This model is similar, at the T-cell level, to recent findings by Warren and Davie (35). They were able to show that high affinity antibody-producing cells are suppressed by carrier-specific suppressor T cells only if the hapten carrier complex is bound to the B-cell surface. In addition, numerous reports have shown that antigenic material shed from cell membranes is bound by immune T cells. For example, Erb et al. (36) reported that macrophages incubated with soluble antigen in vitro release a soluble material which is capable of inducing helper T cells. This material was found to

consist of I-region-coded products and antigenic fragments. Nagy et al. (37) have shown that T cells activated in mixed lymphocyte reactions have either K- or I-region alloantigenic products bound to their surface. Elliott et al. (38) further demonstrated that the bound fragments could be removed from the blast cells by trypsin treatment and, after a recovery period, the cells were able to specifically bind fresh, soluble alloantigens. This finding was recently confirmed and extended by Krammer (39) who showed that soluble alloantigens and anti-idiotypic antibodies compete for the same site on the activated T cells. This site is most likely the specific T-cell receptor.

Summary

Previous studies have shown that suppression of 2,4-dinitrofluorobenzene (DNFB) contact sensitivity by soluble suppressor factor (SSF) requires that the donor of immune lymph node (LN) cells and of SSF share either the H-2K and/or H-2D region of the major histocompatibility complex. Thus, target or acceptor molecules for SSF appear to be coded for by genes within the H-2K and H-2D loci. Experiments were done to investigate the nature of these target molecules and to determine what cell types expressed them. It was found that purified lymph node T cells are suppressed by SSF indicating that T cells express the acceptor molecules. Adsorption experiments showed that the only cells capable of adsorbing the suppressor factor are DNFB-immune T cells from donors which share with the factor-producing strain either the H-2K or H-2D locus. This adsorption can be specifically blocked by pretreating the immune LN cells with antibodies directed against H-2K and/or H-2D determinants or against the hapten DNP but not by antibodies against Ia or θ-antigens. Collectively, these results indicate that the target molecules are expressed only by DNFB-immune T cells and are comprised of histocompatibility antigens associated with DNP.

The author greatfully acknowledges the expert technical assistance of Mrs. Wannell Baird and the secretarial assistance of Ms. Kathy Utschinski. I also thank Dr. Chella David for the generous gift of the antisera and Dr. Henry Claman for helpful discussions during the preparation of this manuscript.

Received for publication 26 July 1979.

References

1. Rich, R. R., and C. W. Pierce. 1974. Biological expressions of lymphocyte activation. III. Suppression of plaque-forming cell responses in vitro by supernatant fluids from concanavalin-A-activated spleen cell cultures. *J. Immunol.* **112:**1360.
2. Zembala, M., and G. L. Asherson. 1974. T cell suppression of contact sensitivity in the mouse. II. The role of soluble suppressor factor and its interaction with macrophages. *Eur. J. Immunol.* **4:**799.
3. Takemori, T., and T. Tada. 1975. Properties of antigen-specific suppressive T cell factor in the regulation of antibody response in the mouse. I. In vivo activity and immunochemical characterizations. *J. Exp. Med.* **142:**1241.
4. Rich, S. S., and R. R. Rich. 1975. Regulatory mechanisms in cell-mediated responses. II. A genetically restricted suppressor of mixed lymphocyte reactions released by alloantigen-activated spleen cells. *J. Exp. Med.* **142:**1391.
5. Thomas, D. W., W. K. Roberts, and D. W. Talmage. 1975. Regulation of the immune response: production of a soluble suppressor by immune spleen cells in vitro. *J. Immunol.* **114:**1616.

6. Tadakuma, T., A. L. Kubner, R. R. Rich, J. R. David, and C. W. Pierce. 1976. Biological expressions of lymphocyte activation. V. Characterization of a soluble immune response suppressor produced by concanavalin-A-activated spleen cells. *J. Immunol.* **117:**323.
7. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T cell factor in the regulation of antibody responses in the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* **144:**713.
8. Thèze, J., J. A. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) extracted from lymphoid cells of nonresponder mice primed with L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). III. Immunochemical properties of the GAT-specific suppressive factor. *J. Exp. Med.* **145:**839.
9. Thèze, J., C. Waltenbaugh, M. E. Dorf, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic acid^{50}-L-tyrosine^{50} (FT). II. Presence of I-J determinants on the GT-suppressive factor. *J. Exp. Med.* **146:**287.
10. Namba, Y., and B. H. Waksman. 1977. Regulatory substances produced by lymphocytes. IV. Further characterization of the inhibitor of DNA synthesis (IDS). *Immunochemistry.* **14:** 143.
11. Greene, M. I., A. Pierres, M. E. Dorf, and B. Benacerraf. 1977. The I-J subregion codes for determinants on suppressor factor(s) which limit the contact sensitivity to picryl chloride. *J. Exp. Med.* **146:**293.
12. Greene, M. I., S. Fujimoto, and A. H. Sehon. 1977. Regulation of the immune response to tumor antigens. III. Characterization of thymic suppressor factor(s) produced by tumor-bearing hosts. *J. Immunol.* **119:**757.
13. Taniguchi, T., and J. F. A. P. Miller. 1978. Specific suppression of the immune response to a factor obtained from spleen cells of mice tolerant to human γ-globulin. *J. Immunol.* **120:** 21.
14. Kontiainen, S., and M. Feldmann. 1978. Suppressor cell induction in vitro. IV. Target of antigen-specific suppressor factor and its genetic relationships. *J. Exp. Med.* **197:**110.
15. Rich, S. S., C. S. David, and R. R. Rich. 1979. Regulatory mechanisms in cell-mediated immune responses. VII. Presence of I-C subregion determinants on mixed lymphocyte reaction suppressor factor. *J. Exp. Med.* **149:**114.
16. 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.
17. Rich, S. S., and R. R. Rich. 1976. Regulatory mechanisms in cell-mediated immune responses. III. I-region control of suppressor cell interaction with responder cells in mixed lymphocyte reactions. *J. Exp. Med.* **143:**672.
18. Rich, S. S., and R. R. Rich. 1976. Regulatory mechanisms in cell-mediated immune responses. IV. Expression of a receptor for mixed lymphocyte reaction suppressor factor on activated T lymphocytes. *J. Exp. Med.* **144:**1214.
19. Tada, T., M. Taniguchi, and K. Okumura. 1977. Regulation of antibody response by antigen-specific T cell factors bearing I region determinants. *In* Progress in Immunology III. T. E. Mandel, C. Cheers, C. Hosking, I. McKenzie, and G. Nossal, editors. Australian Acadamy of Science. Canberra City. 370.
20. Moorhead, J. W. 1977. Soluble factors in tolerance and contact sensitivity to DNFB in mice. I. Suppression of contact sensitivity by soluble suppressor factor released in vitro by lymph node cell populations containing specific suppressor cells. *J. Immunol.* **119:**315.
21. Moorhead, J. W. 1977. Soluble factors in tolerance and contact sensitivity to DNFB in mice. II. Genetic requirements for suppression by soluble suppressor factor. *J. Immunol.* **119:** 1773.
22. Moorhead, J. W. 1978. Tolerance and contact sensitivity to DNFB in mice VIII. Identifi-

cation of distinct T cell subpopulations that mediate in vivo and in vitro manifestations of delayed hypersensitivity. *J. Immunol.* **120:**137.
23. Golub, E. S. 1971. Brain-associated θ antigen: reactivity of rabbit anti-mouse brain with mouse lymphoid cells. *Cell. Immunol.* **2:**353.
24. Pincus, J. H., and R. O. Gordon. 1971. A microassay for the detection of murine H-2 antigens. *Transplantation (Baltimore).* **12:**509.
25. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
26. Davidson, W. F., and C. R. Parish. 1975. A procedure for removing red cells and dead cells from lymphoid cell suspensions. *J. Immunol. Methods.* **7:**291.
27. Sy, M-S., S. D. Miller, J. W. Moorhead, and H. N. Claman. 1979. Active suppression of DNFB-immune T cells. Requirement of an auxiliary T cell induced by antigen. *J. Exp. Med.* **149:**1197.
28. Sy, M-S., S. D. Miller, and H. N. Claman. 1977. Immune suppression with supraoptimal doses of antigen in contact sensitivity. I. Demonstration of suppressor cells and their sensitivity to cyclophosphamide. *J. Immunol.* **119:**240.
29. Rich, S. S., F. M. Orson, and R. R. Rich. 1977. Regulatory mechanisms in cell-mediated immune responses. VI. Interaction of H-2 and non-H-2 genes in elaboration of mixed leukocyte reaction suppressor factor. *J. Exp. Med.* **146:**1221.
30. Schwartz, A., P. W. Askenase, and R. K. Gershon. 1978. Regulation of delayed-type hypersensitivity reactions by cyclophosphamide-sensitive T cells. *J. Immunol.* **121:**1753.
31. Ptak, W., M. Zembala, and R. K. Gershon. 1978. Intermediary role of macrophages in the passage of suppressor signals between T cell subsets. *J. Exp. Med.* **148:**424.
32. Schmitt-Verhulst, A-M., D. H. Sachs, and G. M. Shearer. 1976. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes: confirmation of genetic control of response to trinitrophelyl-modified H-2 antigens by the use of anti-H-2 and anti-Ia antibodies. *J. Exp. Med.* **143:**211.
33. Burakoff, S. J., R. D. Germain, M. E. Dorf, and B. Benacerraf. 1976. Inhibition of cell-mediated cytolysis of trinitrophenyl-derivatized target cells by alloantisera directed to products of the K and D loci of the H-2 complex. *Proc. Natl. Acad. Sci. U.S.A.* **73:**625.
34. Claman, H. N., and S. D. Miller. 1976. Requirements for induction of T cell tolerance to DNFB: efficiency of membrane-associated DNFB. *J. Immunol.* **117:**480.
35. Warren, R. W., and J. M. Davie. 1977. Antigen mediation of a late-acting suppressor T cell activity. *J. Exp. Med.* **146:**1627.
36. Erb, P., M. Feldmann, and N. Hogg. 1976. Role of macrophages in the generation of T helper cells. IV. Nature of genetically related factor derived from macrophages incubated with soluble antigens. *Eur. J. Immunol.* **6:**365.
37. Nagy, Z., B. E. Elliott, and M. Nabholz. 1976. Specific binding of K- and I-region products of the H-2 complex to activated thymus-derived (T) cells belonging to different Ly subclasses. *J. Exp. Med.* **144:**1545.
38. Elliott, B. E., Z. Nagy, M. Nabholz, and B. Pernis. 1977. Antigen recognition by T cells activated in the mixed lymphocyte reaction: specific binding of allogeneic cell material after removal of surface-bound antigen by trypsin. *Eur. J. Immunol.* **7:**287.
39. Krammer, P. H. 1978. Alloantigen receptors on activated T cells in mice. I. Binding of alloantigens and antiidiotypic antibodies to the same receptors. *J. Exp. Med.* **147:**25.