HAPTEN-SPECIFIC T CELL RESPONSES TO 4-HYDROXY-3-NITROPHENYL ACETYL III. INTERACTION OF EFFECTOR SUPPRESSOR T CELLS IS RESTRICTED BY H-2 AND Igh-V GENES*

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The complex regulatory interactions among T lymphocytes initiating, amplifying, and suppressing various effector-immune reactions have recently become the subject of intense investigation. We recently reported on a T cell participating in suppressing the delayed-type hypersensitivity (DTH)[1] response to the haptenic determinant 4-hydroxy-3-nitrophenyl acetyl (NP) (1). We now report on a second population of suppressor T cells capable of modulating the effector phase of the NP-specific DTH response.

NP derivatized syngeneic spleen cells given intravenously induce a population of T lymphocytes that, when administered to animals before sensitization with NP conjugated to bovine gamma globulin (BGG) (NP-BGG), would suppress NP-specific DTH responses elicited with NP conjugated to bovine serum albumin (BSA) (NP-BSA), a heterologous carrier. The fine specificity of these inducer-phase suppressor cells was similar to that of anti-NP antibodies. Thus, these inducer-phase suppressor cells adhered to NP-coated dishes. In addition, in strains of mice bearing the Igh-1^b allotype, the primary anti-NP antibody has a higher affinity for the 5-iodo derivative of NP (NIP) than the original immunizing hapten (2). This property, termed heteroclicity, was found to be demonstrable in the population of NP suppressor cells, i.e., NP-induced suppressor T cells would suppress specifically NIP-BGG-primed, NIP-specific DTH responses in the appropriate strains. The genetic mapping of this particular reactivity demonstrated that the Igh-V gene responsible for heteroclitic idiotypic antibody was closely linked to the gene controlling the NP-induced suppressor cell specificity. In addition, NP-induced suppressor T cells of Igh-1^b origin were specifically lysed by treatment with a guinea pig anti-NP^b idiotype and complement, again demonstrating the expression of Igh-V-gene products in NP-specific suppressor T lymphocytes. In our previous experiments, the NP-specific suppressor T cells were adoptively transferred at the time of immunization and were, therefore, operationally considered afferent suppressor T cells.

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[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DTH, delayed-type hypersensitivity; GAT, poly(L-glu, L-ala, L-tyr); MEM, Eagle's minimum essential medium; MHC, major histocompatibility complex; NIP, 5-iodo derivative of 4-hydroxy-3-nitrophenyl acetyl; NP, 4-hydroxy-3-nitrophenyl acetyl; NP-BGG, NP conjugated to BGG; NP-BSA, NP conjugated to BSA; NP-SC, NP specific suppressor cells; PBS, phosphate-buffered saline; RAMIg, rabbit anti-mouse Ig; T_s, suppressor T cell(s).

In an effort to further investigate the mechanism by which NP-specific suppression operates, cells from NP-suppressed mice were transferred to already primed recipients at various times. When this suppressor spleen cell population was transferred to mice primed at the time of DTH challenge, suppression was still demonstrable. The nature of this efferent suppression and the differential properties of these so-called effector-phase suppressors are the subject of this report.

Materials and Methods

Mice. All mice were either purchased from The Jackson Laboratory, Bar Harbor, Maine, or were bred in the animal facilities of the Harvard Medical School (Boston, Mass.). Mice were used at 2–10 mo of age, and were maintained on laboratory chow and acidified, chlorinated water ad libitum. The B10.AQR(N12) mice were derived from breeding pairs provided by Dr. J. Forman, Southwestern Medical School, Dallas, Tex.

Antigens. BSA and BGG were purchased from Sigma Chemical Co., St. Louis, Mo. NP-*O*-succinimide and NIP-*O*-succinimide were purchased from Biosearch, San Rafael, Calif. The preparation of NP- and NIP-conjugated proteins has been previously described (1, 3). The molar conjugation ratio of haptenic groups used in this work was NP_{17}-BGG, NIP_{18}-BGG,, NP_{11}-BSA, and NIP_9-BSA. The random synthetic terpolymer poly(L-glu, L-ala, L-tyr) (GAT) with an average 40,000 mol wt was custom synthesized by Vega-Fox Biochemicals Div., Newbery Energy Corp., Tucson, Ariz.

Haptenated Cell Preparation. Single cell suspensions of spleen cells were prepared in Eagle's minimum essential medium (MEM) that contained 0.5% heparin. The suspension was pelleted and treated with Tris-NH_4Cl to lyse erythrocytes. After two washes, the spleen cells were resuspended in pH 7.6 phosphate-buffered saline (PBS) at 10^8 cells/ml. 25 μl of a 24 mg/ml solution of NP-*O*-succinimide in dimethylsulfoxide was added per milliliter of cells. After allowing the reaction to proceed for 3 min at room temperature, 10–30 ml of cold, pH 7.4 PBS that contained 1.2 mg/ml glycyl-glycine was added to stop the reaction. The cells were extensively washed in MEM that contained 0.5% heparin before use.

Immunization. Experimental animals were primed with NP-BGG, NIP-BGG, or GAT emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) that contained 2 mg/ml *Mycobacterium butyricum,* so that the final concentration of antigen was 500 μg/ml in the CFA emulsion. 3 d before antigen priming, animals were pretreated with 20 mg/kg cyclophosphamide (Mead, Johnson & Co., Evansville, Ind.) in 0.2 ml PBS injected intraperitoneally. A total of 0.2 ml antigen emulsion was injected subcutaneously, divided between two sites on the dorsal flanks.

DTH Response. 6 d after immunization, mice were challenged for the DTH response by injecting 25 μl of 1 mg/ml of NP-BSA, NIP-BSA, or GAT in PBS into the left footpad with a 27-gauge needle. Footpad swelling was measured 24 h after challenge with an engineer's micrometer (Schlesinger For Tools Ltd., Brooklyn, N. Y.). Swelling was determined as the difference, in units of 10^{-4} in., between the left footpad thickness and the right footpad thickness. All animals were measured by two independent observers, whose measurements were then averaged. The responses thus elicited have been previously demonstrated to be a classical T cell-mediated DTH reaction by the following criteria: (*a*) kinetically the reaction reached a peak at 20–36 h after challenge; (*b*) T cells were required to transfer this reactivity to naive recipients; and (*c*) major histocompatibility complex (MHC) identity at the I-A subregion was necessary for transfer of reactivity (3).

Antisera. Rabbit anti-mouse Ig (RAMIg) was prepared as follows: After several immunizations of a rabbit with purified normal mouse Ig, the rabbit antisera was passed over an immunoadsorbent that was prepared by coupling a 40% saturated ammonium sulfate precipitate of normal mouse serum to Sepharose 4B (Pharmacia Fine Chemicals Inc., Uppsala, Sweden). The RAMIg was eluted from the column with 0.1 M glycine-HCl solution at pH 2.8, neutralized in 2 M Tris buffer at pH 7.9, dialyzed against PBS, and adjusted to a final protein concentration of 1 mg/ml.

Guinea pig anti-NPb anti-idiotypic antisera were prepared with affinity-purified B6 anti-NP antibodies obtained from primary sera of a pool of C57BL/6 mice immunized with 100 μg

NP-BGG in alum with 0.1 ml pertussis vaccine (U. S. Department of Public Health, Ann Arbor, Mich.). The antibodies were purified by precipitation with 45% saturated ammonium sulfate followed by affinity purification on an NP-BSA-coupled Sepharose 4B immunoadsorbent. Specific antibody was eluted from the column by washing the column with a 0.03 M NIP-caproate (Biosearch) in PBS solution. The eluate was extensively dialyzed in PBS. The eluate from this column contained λ_1-light chain and μ-, γ_1-, and small amounts of γ_{2a}-heavy chains as detected by radioimmunodiffusion (4). Furthermore, isoelectric focusing of a sample of the radioiodinated purified Ig in a pH 3–10 range polyacrylamide gel revealed a remarkably restricted spectrotype. Guinea pigs were immunized biweekly with 200 μg of purified C57BL/6 anti-NP antibodies in CFA. 2 wk after the third injection, the animals were bled. The antisera were adsorbed on NP conjugated to the synthetic polymer of L-glutamic acid and L-lysine, MOPC 104E (μ- and γ_1-heavy chains), and normal mouse serum immunoadsorbents prepared by glutaraldehyde cross-linking. MOPC 104E specifically purified antibody was the kind gift of Dr. Abul Abbas (Dept. of Pathology, Harvard Medical School). These gels were prepared according to the procedure of Avrameas and Ternynck (5). After exhaustive adsorptions on these gels, the specificity of this reagent was verified with an inhibition of idiotype binding assay, according to the method of Ju et al. (6). 30 μl of normal mouse sera, MOPC 104E ascites, 5 μl A/J anti-NP sera, and 1.25×10^{-4} M DNP-caproate did not inhibit idiotype binding. However, 1 μl of primary B6 anti-NP sera, 4.25×10^{-6} M NP-caproate or 2.0×10^{-6} NIP-caproate could significantly inhibit the idiotype binding. Thus, the anti-idiotypic antiserum used detects combining site determinants as previously described by other groups (7, 8).

Cell Fractionation. T cells were purified according to Mage et al. (9). 5 ml of a 1 mg/ml solution of purified RAMIg was added to 100- × 15-mm polystyrene Petri plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) and incubated for 1 h at room temperature. Unbound antibody was removed, and the plates were then washed extensively in PBS that contained 5% fetal calf serum. Spleen cells, previously treated for 3 min at 37°C with Tris-NH_4Cl to lyse erythrocytes, were resuspended at a concentration of 1.2×10^7 cells/ml. 6 ml of cells was added to the antibody-coated plates and incubated for 1 h at 4°C. The nonadherent T cell population was recovered by gentle swirling.

Antisera Treatments. Whole spleen lymphocyte populations of SJL strain mice were treated with anti-idiotypic antiserum and complement. A maximum of 3×10^8 cells were pelleted in a 17 × 100-mm plastic tube (Falcon Labware, Div. of Becton, Dickinson & Co.), and resuspended in 0.5 ml of unabsorbed anti-idiotypic antiserum. The suspension was incubated at room temperature for 30 min. The antisera was then washed out, and the cells were resuspended in 1.5 ml of prescreened low-toxicity rabbit complement. This suspension was incubated at 37°C for 45 min, the cells then washed three times, counted, and transferred to the appropriate recipients. Anti-Thy-1.2 treatments were performed as detailed elsewhere (3).

Results

Cellular Requirement for Transfer of Effector-Phase Suppression. To induce NP-specific suppressor cells, a suspension of erythrocyte-free spleen cells was derivatized with NP-*O*-succinimide. 2×10^7 NP-derivatized spleen cells were injected intravenously into syngeneic mice. After 7 d, these mice were sacrificed, and their spleen cells (suppressor cells) were transferred to syngeneic recipients that were previously cyclophosphamide treated and primed. These recipients had been pretreated with 20 mg/kg cyclophosphamide 3 d before immunization with 100 μg NP-BGG in CFA. 6 d after NP-BGG priming, the animals received 4×10^7 control or suppressor spleen cells (NP-SC). 2–4 h after transfer, the mice were challenged by injection of 25 μg NP-BSA in 25 μl of PBS into the left footpad. The DTH response was measured as the increment of footpad swelling 24 h after challenge of the left foot as compared with the uninjected right foot.

Table I indicates that SJL and C57BL/6 mice, when given spleen cells on the day of DTH challenge, from animals previously injected with NP-derivatized spleen cells,

TABLE I

*T Lymphocytes Mediate Effector-Phase Suppression**

Strain	Immunogen	Cells transferred	NP DTH response
SJL	NP-BGG	4×10^7 control	45.7 ± 2.0
		4×10^7 NP-SC‡	13.8 ± 2.5§
		4×10^7 NP-SC + NMS + C	7.9 ± 3.0
		4×10^7 NP-SC + anti-Thy + C	38.6 ± 2.3§
	CFA	None	5.5 ± 1.6
C57BL/6	NP-BGG	4×10^7 control	65.4 ± 1.2
		4×10^7 NP-SC	24.1 ± 2.8§
		12×10^6 NP-Ts	27.6 ± 3.4§
		6×10^6 NP-Ts	44.6 ± 6.9
	CFA	None	13.4 ± 2.0

* Groups of four to five mice were pretreated with 20 mg/kg cyclophosphamide 3 d before priming with 100 μg NP-BGG in 200 μl of CFA, that was administered in divided doses subcutaneously in both flanks. 6 d later, groups of mice received the indicated cell populaltions intravenously. On the same day as the cell transfer, the experimental animals were challenged with 25 μg of NP-BSA in 25 μl of PBS in the left footpad, to determine an NP-specific DTH response. The DTH response was measured 24 h after challenge and was determined as the net footpad swelling elicited by the injected compound, in units of 10^{-4} in.

‡ NP-SC are spleen cells from mice receiving 2×10^7 NP-derivatized syngeneic spleen cells 7 d before use (Materials and Methods). NMS, normal mouse serum; C, complement.

§ $P < 0.05$ as compared with the appropriate control group.

were unable to mount a strong NP-specific DTH response. Treatment of NP-SC populations with anti-Thy-1.2 antiserum plus complement abrogated the ability of the cells to transfer suppression, which indicated that T cells are necessary for the transfer of this effector-phase suppression. To determine that T cells alone were able to transfer the suppression, T cells were purified from NP-suppressor cell populations by negative selection with RAMIg-coated Petri dishes. These cells contained <5% Ig-positive cells by immunofluorescence. Recovery was ~25%. Either 12×10^6 or 6×10^6 of this enriched T cell population was then transferred to primed mice on the day of challenge. As shown in Table I, 12×10^6 purified suppressor T cells (T_s) were sufficient to mediate nonresponsiveness. These two experiments demonstrate that T cells are both necessary and sufficient to transfer the observed suppression. Because of the ability of these cells to acutely suppress an already primed response, this population of T cells was termed effector suppressor cells.

Specificity of NP-induced Effector Suppressor Cells in C57BL/6 Mice. C57BL/6 mice were primed with NP-BGG, NIP-BGG, or a completely unrelated synthetic terpolymer, GAT. 6 d after priming, groups of mice received 4×10^7 control or NP-induced effector suppressor cells. As indicated in Table II, the suppressor cell population, although efficiently suppressing the NP-specific DTH response, had no effect on the GAT-specific DTH response.

Because it had been previously demonstrated that NP-induced suppressor T cells of C57BL/6 origin, when given to mice on the day of priming, could also suppress the NIP-specific DTH response (1), the effect of NP-induced effector suppressors on NIP-specific DTH responses was examined. As seen in Table II, NP-induced effector suppressor cells were marginally suppressive on NIP-specific responses. The magnitude of the suppression was ~30% ($P = 0.04$). This small degree of NIP-specific suppression has been a consistent finding in a number of other experiments, in which the degree of suppression ranged from 5 to 40%.

TABLE II

*Specificity of NP-induced Effector Suppressor Cells in C57BL/6 Mice**

Immunogen	Challenge	Cells transferred	
		Control	NP-SC
NP-BGG	NP-BSA	49.8 ± 3.8	13.1 ± 2.2‡
CFA	NP-BSA	7.2 ± 2.8	
NIP-BGG	NIP-BSA	51.2 ± 4.5	36.1 ± 8.1‡
CFA	NIP-BSA	6.3 ± 3.0	
GAT	GAT	66.1 ± 8.5	75.6 ± 9.5
CFA	GAT	29.5 ± 7.2	

* See Table I.

‡ $P < 0.05$ as compared with the appropriate control group.

TABLE III

*Anti-Idiotype Treatment of Inducer and Effector Suppressor Populations in SJL Mice**

Immunogen	Cells transferred	Time of cell transfer	
		Day 0	Day 6
NP-BGG	Control	46.3 ± 4.9	46.3 ± 4.4
	NP-SC‡	5.9 ± 3.8§	16.7 ± 3.3§
	NP-SC + NGPS + C‖	9.8 ± 3.4	18.9 ± 4.4
	NP-SC + anti-NP^b + C	36.2 ± 3.4§	16.1 ± 7.5
CFA	—	3.9 ± 1.2	6.2 ± 4.9

* 4×10^7 splenic NP suppressor cells were given each recipient. These cells or control SC were transferred to mice either before immunization with NP-BGG (Day 0), or 6 d after immunization with the antigen (Day 6). On day 6, mice were challenged with NP-BSA as described in Materials and Methods. DTH response was measured 24 h after challenge as the net footpad swelling.

‡ See Table I.

§ See Table I.

‖ NGPS, normal guinea pig serum; anti-NP^b, guinea pig anti-NP^b idiotype; C, complement.

Anti-Idiotype Treatment of NP-induced Suppressor Cell Population. Because of the apparent difference in specificity of suppression observed when mice were given suppressor cells on the day of priming as opposed to day of challenge, it was apparent that different suppressor cell populations might be responsible for the two phenomena. SJL mice were given 2×10^7 NP-derivatized syngeneic spleen cells intravenously. 7 d later, these mice were sacrificed and 4×10^7 suppressor cells were then transferred to recipients that had been primed with NP-BGG 6 d earlier, or to mice that were primed after the cell transfer. Thus, the NP-SC population was given at either the effector or induction phases of the immune response (Table III). Additional groups of mice received these suppressor cells after in vitro treatment with guinea pig anti-idiotype (anti-NP^b) or normal guinea pig serum plus complement. The anti-idiotype used was raised by immunization of a guinea pig with a pool of specifically purified C57BL/6 primary anti-NP antibodies. The characterization of this antiserum is described in Materials and Methods. The anti-idiotype-treated NP-induced suppressor cell populations lost the ability to suppress the afferent mode of the NP-primed, NP-elicited DTH response. The same treated cell population, however, retained the ability to suppress such NP-specific responses when given to already primed animals

on the day of challenge. Thus, the differential susceptibility of the effector suppressor to anti-idiotype plus complement define a second NP-specific T_s population in the original splenic population.

H-2 Restriction of Transfer of NP-Specific Effector Suppression. To investigate the role of the MHC in the ability to transfer NP-specific effector suppression, allogeneic transfer experiments were performed with a series of H-2 congenic mice (Table IV). B10 and 4R donors of effector suppressor cells could transfer NP-specific effector suppression into H-2-identical hosts; however, they would not transfer suppression to strains that differed from the donor strain at the I-A region of the H-2 complex (Table IV). The inability to transfer suppression allogeneically was not a result of acute rejection by the recipients, because (B6 × C3H)F_1 effector suppressors were operative in a B10 host.

To determine the critical subregions required for transfer, 4R effector suppressors were transferred into B10.AQR. The latter strain shares H-2 homology with the 4R donor strain only at the I-A subregion of the H-2 complex. Significant levels of suppression were transferred between these strains. Thus, I-A homology alone is sufficient for transfer of NP-specific effector suppression. All the other data are consistent with this hypothesis.

Allotype-linked Restriction on Transfer of NP-Specific Effector Suppression. While performing the above experiments, we had several indications that the ability to transfer effector suppressor cells was not solely controlled by the H-2 complex. Thus, we noted that it was not possible to transfer NP effector suppressor cells between H-2-identical strains that differed in their non-H-2 background. The data in Table V demonstrate that genes in the Igh-V region can also restrict the ability to transfer NP-SC.

NP-specific effector suppressors were transferred between the BALB/c and C.B-20 strains, on the day of challenge for an NP-specific DTH response. As indicated in Table V, the BALB/c NP effector suppressors did not have an effect on allotype congenic C.B-20 mice; similarly the C.B-20 suppressor population did not suppress BALB/c hosts. This indicates that Igh genes control the ability of the NP effector T_s to transfer suppression. The BAB/14 strain was examined because it carries a recombinant Igh allele in which crossing-over occurred between the Igh-C and -V genetic regions. This strain has the Igh-C allele of C.B-20 and the Igh-V region

TABLE IV

*Transfer of Effector Suppression is Restricted by H-2 Genes**

Strain	H-2 formulae (K,A,B,J,E,C,S,D)	Cells transferred					
		B10 control	B10 NP-SC‡	4R control	4R NP-SC	(B6 × C3H)F_1 control	(B6 × C3H)F_1 NP-SC
B10	bbbbbbbb	40.0 ± 1.8	11.4 ± 2.7§	50.4 ± 3.2	52.0 ± 4.6	46.3 ± 2.5	12.3 ± 2.7§
4R	kkbbbbbb	40.4 ± 6.9	38.6 ± 7.7	43.3 ± 3.9	3.9 ± 3.6§		
5R	bbbkkddd	40.7 ± 2.9	17.3 ± 3.8§				
3R	bbbbkddd	43.3 ± 2.0	18.5 ± 2.8§				
B10.GD	ddbbbbbb	41.3 ± 2.4	39.8 ± 2.0				
B10.A	kkkkkddd			49.3 ± 3.7	12.8 ± 1.7		
B10.AQR	qkkkkddd			51.2 ± 7.9	20.3 ± 6.3§		
6R	qqqqqqqd			41.3 ± 2.6	44.3 ± 4.9		

* See Table I.
‡ See Table I.
§ See Table I.

TABLE V

*Transfer of Effector Suppression is Restricted by Igh-V Genes**

Recipient	Cells transferred	NP DTH response
C.B-20	Control	41.7 ± 2.4
	BALB/c NP-SC‡	41.3 ± 5.0
	C.B-20 NP-SC	7.1 ± 3.4§
BALB/c	Control	39.4 ± 2.8
	BALB/c NP-SC	14.2 ± 4.6§
	C.B-20 NP-SC	44.1 ± 4.9
BAB/14	Control	48.2 ± 3.3
	BALB/c NP-SC	12.8 ± 1.0§
	C.B.-20 NP-SC	42.0 ± 5.2
BALB.B	Control	38.2 ± 1.6
	BALB/c NP-SC	41.7 ± 0.9
	C.B-20 NP-SC	36.2 ± 4.0

* See Table I.
‡ See Table I.
§ See Table I.

(controlling anti-NP responses) of BALB/c. BALB/c suppressors could suppress BAB/14 NP-specific DTH responses. Thus, Igh-C homology is not required, and Igh-V homology is sufficient for elicitation of the suppressive effects. When C.B-20 strain suppressors were transferred to BAB/14, no significant suppression was observed. This indicates that Igh-C homology alone is insufficient for the transfer of effector-phase suppressor cells. Taken together, these experiments indicate that an Igh-V match is required between the donor and recipient to obtain effector suppression.

To establish that the ability to transfer NP effector suppressor cells is restricted by both Igh-V and MHC genes, we tested the ability of BALB/c NP-SC to transfer suppression to the BALB.B strain, which shares common Igh alleles but differs at the H-2 complex. As shown in Table V, we could not transfer NP effector suppressor cells in this combination. Thus, for the successful transfer of effector suppressor cells there must be donor-host compatibility for both MHC and Igh-V genes.

Discussion

The experiments described demonstrate the ability of intravenously administered NP-derivatized syngeneic spleen cells to induce a population of NP-specific T_S capable of mediating suppression when given to previously primed animals (Tables I and II). Because of this capability, such T_S are termed effector-phase T_S, or, for simplicity, effector suppressors. The T cell nature of this phenomenon was demonstrated, i.e., T cells were shown to be both necessary and sufficient to mediate such suppression. The existence of effector-phase T_S active on cell-mediated immune responses was previously demonstrated in the DNP contact-sensitivity system (10). Furthermore, Asherson et al. (11, 12) reported that a T cell-derived factor could be obtained from mice given an intravenous injection of picryl sulfonic acid; this factor, when cotransferred with lymphocytes from mice primed with picryl chloride, suppressed the transfer of contact sensitivity. The observations herein extend the phenomenon to another, distinct cell-mediated immune response–DTH to haptenic determinants on soluble protein carriers.

The specificity of the NP effector T_S contrasts with that found for the suppression produced by transfer of the suppressive populations before priming. To distinguish the two protocols, and, as will be argued below, the two cells involved, we have termed these, respectively, induction-phase and effector-phase suppressors. The NP induction-phase suppressors in C57BL/6 mice were shown to suppress NIP-primed responses with greater efficiency than even NP-primed responses (1). In contrast, NP effector-phase suppression in C57BL/6 mice was only weakly cross-reactive on the NIP-primed DTH response.

The difference in the specificity of suppression noted in the NP induction- and effector-phase suppressors led us to search for a phenotypic difference between the cells. The differential susceptibility of the induction and effector suppressors to anti-idiotype plus complement treatment indicated that the two modes of suppression involved distinct T cells. Furthermore, preliminary experiments indicate that these two cells are physically separable on the basis of their antigen or idiotype binding specificity. Thus, the inducer suppressor cell bears an idiotypic receptor, whereas the effector suppressor cell apparently bears an anti-idiotypic receptor. It should be emphasized that these two cell populations are simultaneously present in the same animal.

The involvement of two T cells in the suppression of immune responses has been demonstrated for several other systems. Waltenbaugh et al. (13) first demonstrated the ability of a suppressor factor derived from T cells, T_sF, to induce polypeptide-specific T_S, termed T_{s2}. The distinctiveness of these two cells was convincingly shown by demonstration of strains capable of making T_{s2} with exogenous T_sF, but themselves unable to produce T_sF or T_{s2}. This has been confirmed in the azobenzenearsonate system (14). Another example of a multicellular pathway that effects suppression is the model proposed by Eardley et al. (15, 16) of T cell-dependent feedback suppression of an ongoing immune response. These investigators showed that in an in vitro anti-erythrocyte response, an Ly-1^+, I-J^+, Qa-1^+ T cell acted upon an Ly-1^+,2,3^+, I-J^+, Qa-1^+ T cell leading to production of an Ly-23^+ T_S, which itself could dampen both the Ly-1^+, I-J^+ inducer and the Ly-1^+, I-J^- helper T cell activity. The relationship between the NP-induced, inducer and effector T_S has not been addressed in the studies reported here.

The presence of both the effector and inducer T_S populations in spleens derived from mice given NP-derivatized syngeneic cells raises an interesting question, i.e., whether the effector T_S can operate in the absence of the inducer T_S, or if purified effector suppressors active at the time of antigen priming might display a phenotypically different specificity than the same cells transferred to an already primed recipient. That the latter may indeed by the case was suggested by the experiments shown in Table V. These experiments demonstrate that Igh-V homology is required between donor and recipient for efficient operation of NP-effector T_S. Because of the lack of detectable idiotype on the effector T_S (Table III), a reasonable and likely hypothesis is that the NP effector T_S recognizes the Igh-V gene product expressed by the T_{DTH} or some other T cell critical in expression of DTH, i.e., the receptor on the NP effector T_S is anti-idiotypic in nature. These findings are in agreement with the data of Owen et al. (17), Bona and Paul (18), and Sy et al. (14), who demonstrated that T_S can express anti-idiotypic receptors. Preliminary experiments alluded to earlier suggest that this is also the case for NP effector suppressor cells. This provides

strong genetic support for basic assumptions of network theories (19). The feedback loop described by Eardley et al. (16) also shows Igh-V restriction at the level of the Ly-1^+, I-J^+, Qa-1^+ suppressor inducer interaction with the Ly-1^+,$2,3^+$ intermediate T cell.

The data in Tables IV and V also demonstrate that the NP effector suppressor is also H-2 restricted. Mapping of the required subregion indicates that I-A homology is sufficient for transfer of suppression, in the absence of other non-H-2 homology. The sufficiency of K-end homology could not be determined with the strains of mice available. Miller (20) recently reported a D-end restriction on the function of suppressor in contact sensitivity to dinitrofluorobenzene. Aside from these reports, MHC restriction in the pathway to suppression of immune responses has been investigated at the level of various T_S-derived factors (21, 22). It may be argued that these latter situations are not analogous to the one investigated here in that MHC restriction may involve restriction on the activation of factor production or the ability to accept such factors.

Whether the observed MHC restriction is mediated by the same cell in the NP effector suppressor populations, which is also Igh-V restricted, or if this represents another level of subset specificity mediated by different cells cannot be determined from experiments done thusfar. Teleologically, it would seem more likely that the same cell would mediate both effects. A simultaneous MHC and receptor-anti-receptor recognition would add another level of complexity to the posulated network of lymphocyte interactions mediating immune responses and regulation.

Summary

4-Hydroxy-3-nitrophenyl acetyl (NP)-derivatized syngeneic spleen cells administered intravenously induced a population of suppressor T cells that could suppress mice previously primed to NP. The effect was demonstrable when the suppressor cells were transferred to NP-primed mice on the day of challenge for delayed-type hypersensitivity (DTH) responses. In contrast to the suppressor T cell population, which abrogates 5-iodo derivative of NP (NIP)-specific DTH responses when administered before antigen priming, the effector-phase suppressors did not efficiently suppress NIP-specific DTH responses, and were not lysed by treatment with anti-idiotype plus complement. Adoptive transfer experiments between major histocompatibility complex and allotype congenic strains of mice allowed demonstration of both Igh-V and I-A restrictions in the transfer of this cell population. The implications of these data in terms of network theories and proposed cellular models for negative immunoregulation were discussed.

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