HAPTEN-SPECIFIC T CELL RESPONSES TO 4-HYDROXY-3-NITROPHENYL ACETYL

IX. Characterization of Idiotype-specific Effector-Phase Suppressor Cells on Plaque-forming Cell Responses In Vitro*

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It has been shown in several systems that the manifestation of antigen-specific immune suppression involves the interactions of suppressor T cell subsets (1–8). In some instances, these interactions depend on the recognition of unique idiotypic determinants present on lymphoid cells (6, 9–12), or regulatory molecules derived from these cells (13–15). Thus, these T cell interactions appear to require idiotypic recognition in a fashion analogous to the antibody network originally proposed by Jerne (16).

Previously, we have described a suppressor cell system in which suppressor T cells specifically affect idiotype-bearing B lymphocytes both in vivo and in vitro (17). In contrast to several systems in which idiotype-specific suppression has been induced by the injection of anti-idiotypic antiserum (10, 15, 18) or idiotype itself (11, 19, 20), suppression in this system was induced directly with antigen. Spleen cells were covalently coupled with the 4-hydroxy-3-nitrophenyl acetyl (NP)[1] hapten and injected intravenously into syngeneic recipients. Spleen cells from these recipients specifically suppress NP^b idiotype-bearing B cell clones. That is, spleen cells from donors treated with NP-coupled cells were capable of suppressing the NP^b idiotypic response of NP-primed B cells when added at the initiation of in vitro culture. The idiotype specificity of these suppressor cells suggested that they interacted with target cells by recognition of idiotypic determinants. Based on these observations, it was hypothesized that the suppressor cell described in vitro may correspond to the effector-phase suppressor cell (Ts^e) involved in the suppression of NP delayed-type hypersensitivity responses (6). To test this hypothesis and to characterize this cell population further, suppressor cells were examined in vitro for their ability to act in the effector phase of an immune response, and for the genetic restrictions on their activity. The data presented herein indicate that NP-induced immune suppression of a B cell response is mediated, at least in part, by an idiotype-binding $Lyt\text{-}1^-$, $Lyt\text{-}2^+$ T cell that can function during the effector phase of an immune response. The results are consistent with the

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[1] *Abbreviations used in this paper:* ABA, arsinilate; BGG, bovine gammaglobulin; C, complement; DTH, delayed-type hypersensitivity; GLϕ, poly-(L-Glu^{56}-L-Lys^{35}-L-Phe^{9}); KLH, keyhole limpet hemocyanin; MEM, minimum essential medium; NP, 4-hydroxy-3-nitrophenyl acetyl hapten; PFC, plaque-forming cells; RAMIg, rabbit anti-mouse immunoglobulin; SRBC, sheep erythrocytes.

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contention that this cell population may be involved in the regulation of both T and B cell immune responses.

Materials and Methods

Mice. C57BL/6 male and C3H/HeJ male mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. C3H.NB and CKB male mice were bred in the Harvard Medical School facilities. All experimental animals were between 8 and 12 wk of age at the beginning of immunization. Animals were age-matched in each experiment.

Antigens. NP_{22}-bovine gammaglobulin (BGG), NP_6-poly-(L-Glu^{56}-L-Lys^{35}-L-Phe^9) (GLϕ), NP-keyhole limpet hemocyanin (KLH), and NP-Ficoll were conjugated by the reaction of NP-*O*-succinimide (Biosearch, San Rafael, Calif.) with GLϕ (lot GLP1-71-199; Miles-Yeda Ltd., Rehovot, Israel), KLH, BGG (Sigma Chemical Co., St. Louis, Mo.), or $AECM^{75}$-Ficoll (Biosearch) as described previously (6). The NP-KLH and NP-Ficoll had an average of 30 and 17 NP groups per 100,000 mol wt, respectively.

Immunization. Mice were immunized intraperitoneally with 150 μg NP-KLH in a 0.2-ml mixture containing 25% pertussis vaccine (Michigan Dept. of Public Health, Lansing, Mich.).

Haptenated Cell Preparation. NP-coupled spleen cells were made as described previously (17). Cells were washed extensively in minimum essential medium (MEM) before intravenous injection of 3×10^7 NP-coupled cells into syngeneic recipients.

In Vitro Subcultures. Spleen cells from control mice or mice treated 7 d earlier with NP-coupled spleen cells were teased and washed under sterile conditions. These cells were added to Linbro culture wells (Flow Laboratories, Inc., Hamden, Conn.) at a concentration of 7.5×10^6 cells/ml/well in Mishell-Dutton culture medium containing 10% fetal calf serum, (FCS; lot 95045, Microbiological Associates, Walkersville, Md.). Cultures were incubated at 37°C in a 10% CO_2 atmosphere in rocking culture boxes and fed daily with 0.1 ml supplementary medium for a 4-d subculture period. Cells were then pooled, washed, and 5×10^5 viable normal or suppressor cells were added to 7.5×10^6 NP-primed cells.

Purification of T Cells and Fractionation of NP^b Idiotype-binding Cells. The method of Mage et al. (21) was used to purify splenic T cells from subculture. Purified rabbit anti-mouse immunoglobulin (RAMIg; 5 ml of a 1 mg/ml solution) was added to 100- × 15-mm polystyrene petri plates (1005; Falcon Plastics, Div. of Becton, Dickinson & Co., Oxnard, Calif.) and incubated for 1 h at room temperature. Unbound antibody was washed off and 8×10^7 viable cells from subcultures were added for a 1-h incubation period at 20°C. The nonadherent T cell population was recovered by gentle swirling. Less than 1.0% of these cells stained with a fluorescent RAMIg antiserum. Idiotype-specific suppressor cells were separated according to the method of Abbas et al. (22). Anti-NP antiserum was prepared by immunization of C57BL/6 (Igh-1^b) or C3H.NB (Igh-1^j) mice with 100 μg NP-BGG in pertussis vaccine and specific antibody was obtained by affinity purification. 5 ml of a 1 mg/ml solution of purified anti-NP antibodies from C57BL/6 (Igh-1^b) or C3H.NB (Igh-1^j) mice were incubated on 100- × 15-mm polystyrene petri dishes for 1 h at room temperature. After removal of immunoglobulin solutions, the plates were washed as above. 10×10^6–15×10^6 purified suppressor T cells were added to each plate in a total volume of 5 ml MEM containing 5% FCS. Cells were incubated for 1 h at room temperature. Nonadherent cells were removed and the dishes were washed extensively with warm (20°C) media. After addition of 5.0 ml cold media, the dishes were incubated on ice for 30 min. Adherent cells were resuspended with vigorous pipetting, washed, and counted. 1.0–5×10^5 viable fractionated cells were then added to responder cultures challenged 4 d earlier with 100 ng NP-Ficoll or NP-GLϕ.

Anti-Lyt Treatment. 5×10^6 suppressor cells from subcultures were washed twice in MEM without FCS, pelleted, and incubated for 45 min at 20°C with 2–15 μl monoclonal, arsinilate (ABA)-conjugated anti-Lyt-1 or anti-Lyt-2 antibody (Becton, Dickinson & Co., Oxnard, Calif.). Cells were washed twice and resuspended in 15 μl rabbit anti-ABA antibody (Becton, Dickinson & Co.) and incubated for 45 min at 20°C. Treated cells were then washed and incubated in 0.3 ml rabbit complement (C) diluted 1:5 in MEM containing 1% DNase. After a 30-min incubation at 37°C, the cells were washed in MEM and 1.8–3.5×10^5 viable cells were added to responder cultures challenged 4 d earlier with NP-Ficoll. Control cells were treated only with anti-ABA antibody and C.

In Vitro Responder Cultures. C57BL/6 mice were immunized intraperitoneally with 150 μg NP-KLH with 25% pertussis vaccine. 4 wk later, their spleens were removed and teased into a single cell suspension under sterile conditions. Cells were washed and resuspended in Mishell-Dutton media containing 10% FCS. 7.5×10^6 viable NP-primed cells was added to each well of Linbro culture plates. Cultures were challenged with 100 ng NP-GLϕ or NP-Ficoll in 20 μl. A primary sheep erythrocyte (SRBC) response was obtained by addition of 2×10^6 washed SRBC to culture wells containing 7.5×10^6 normal, unprimed spleen cells. All cultures were incubated in rocking boxes at 37°C in a 10% CO_2 atmosphere and were fed daily with 0.1 ml supplementary medium. 4 d after challenge, subcultured control or suppressor cells were added. 5 d after challenge, duplicate wells were pooled and assayed for total NP- or SRBC-specific plaque-forming cell (PFC) responses in replicate slides.

PFC Assay. NP- or SRBC-specific PFC were assayed as reported previously (17). NP^b-bearing B cells were inhibited from forming plaques by the addition of 3.0–4.0 μl anti-idiotypic reagent to each slide at the beginning of the assay. The control response was determined by addition of 3.0–4.0 μl normal guinea pig gammaglobulin. The percent inhibition of PFC was calculated according to the formula:

$$\text{Percentage of inhibition} = \left(1 - \frac{\text{experimental PFC}}{\text{control PFC}}\right) \times 100.$$

Anti-NP^b Antiserum. Anti-idiotypic antiserum specific for the NP^b idiotype was prepared as described previously (23). This antiserum has been shown to specifically inhibit NP^b idiotype-positive B cells in a plaque assay system (17).

Results

Idiotype-specific Suppression in the Effector Phase. In a previous report (17), we have shown that after a 4-d subculture, spleen cells from mice treated 7 d earlier with the intravenous injection of NP-coupled syngeneic spleen cells were capable of suppressing the expression of NP^b idiotype-positive B cells when added to responder cultures at the time of antigen challenge. Spleen cells from NP-spleen-cell-treated donors that were not subcultured for a 4-d period failed to suppress the in vitro response. These data suggested that a population of effector-phase suppressor cells matured during the subculture period. To determine whether suppressor cells were capable of affecting the expression of NP^b idiotype-positive B cells during the effector phase of the response, mice were given an intravenous injection of 3×10^7 syngeneic control or NP-modified spleen cells. 7 d later, spleen cells were subcultured for a 4-d period, after which 5×10^5 viable control or suppressor cells were added to NP-KLH primed responder cultures that had been challenged in vitro 4 d earlier with NP-Ficoll. On the following day, the cultures were assayed for the magnitude and the idiotype content of the response. The percent idiotype-positive B cell clones was assayed by the ability of an NP^b idiotype-specific guinea pig antiserum to inhibit the formation of plaques. The data in Table I confirm previous experiments demonstrating the expression of 75–100% idiotype-positive B cells in cultures challenged with NP-GLϕ or NP-Ficoll. The data also indicate that subcultured suppressor cells were capable of significantly decreasing the magnitude of immune responsiveness when added late after antigenic challenge (Table I). Furthermore, this suppression appeared to be idiotype specific because the response of cultures to which suppressor cells had been added was essentially nonidiotypic. Thus, subcultured cells derived from NP-spleen-cell-treated donors specifically suppressed the NP^b idiotypic response in the effector phase of these in vitro responses.

Suppressor Cells Specifically Adhere to NP^b Idiotype. Because the addition of subcul-

TABLE I

*NP^b Idiotype-specific Suppression of Effector-Phase Responses In Vitro**

Antigen	Donor treatment	NP-specific PFC/culture $\overset{\times}{\div}$ log SE‡	Percent inhibition with anti-NP^b antiserum ± SE§
NP-GLϕ	Control	11,000	75
	NP-spleen	3,400 (66)	0
NP-Ficoll	Control	3,200 $\overset{\times}{\div}$ 1.4	100 ± 0
	NP-spleen	1,300 $\overset{\times}{\div}$ 1.1 (59)	7 ± 7

* C57BL/6 mice received an intravenous injection of 3×10^7 control or NP-coupled syngeneic spleen cells. 7 d later, those spleen cells were cultured for a 4-d period. 5×10^5 viable control or suppressor cells from these subcultures were then added to responder cultures challenged 4 d earlier with 100 ng NP-Ficoll or NP-GLϕ. 1 d later, duplicate wells were pooled and assayed for direct (NP-Ficoll) or indirect (NP-GLϕ) PFC responses. Data from one experiment with NP-GLϕ and two experiments with NP-Ficoll are presented.

‡ The data are expressed as the geometric mean $\overset{\times}{\div}$ log SE. The percent suppression relative to controls is presented in parentheses.

§ The percent idiotype-positive response is expressed as the arithmetic mean ± SE of the percent inhibition of plaque formation in the presence of 3.0–4.0 μl anti-NP^b antiserum relative to the response in the presence of normal guinea pig IgG.

tured cells from NP-modified-cell-treated donors resulted in the suppression of NP^b idiotype-positive B cell clones, we next tested whether this suppressor cell population carried an anti-idiotypic receptor. Subcultured suppressor cells were T cell-enriched by passage on RAMIg-coated petri dishes. These T cells were subsequently passed on petri dishes coated with specifically purified anti-NP antibody either from C57BL/6 mice ($Igh\text{-}1^b$), which bear the NP^b idiotype, or from C3H.NB mice ($Igh\text{-}1^j$), which do not bear the NP^b idiotype. Viable cells thus fractionated were added in various concentrations to responder cultures challenged 4 d earlier with NP-Ficoll. The results from two representative experiments are presented in Table II. Purified suppressor T cells mediate this immune suppression because RAMIg nonadherent subcultured cells from NP-tolerized donors suppress the effector-phase immune response (Table II). In addition, these suppressor T cells bound to NP^b idiotype-coated plates because as few as 1×10^5–3×10^5 idiotype adherent cells suppressed the NP response 46–100% in the effector phase, whereas the idiotype nonadherent fraction was completely depleted of suppressor activity. That the suppressor cells did not recognize NP hapten potentially bound by the purified antibody was demonstrated by the inability of cells adherent to plates coated with C3H.NB anti-NP antibody to affect the response. As expected, all of the suppressive activity was found in the C3H.NB anti-NP antibody-coated plate nonadherent fraction. Furthermore, in two experiments (data not shown) suppressor cells failed to bind to NP-bovine serum albumin-coated petri dishes. The results indicate that subcultured cells from NP-modified spleen-cell-treated donors were T cells that specifically recognized NP^b idiotypic determinants and did not directly function by an antigen-bridging mechanism.

Igh Homology Is Required to Transfer Suppression In Vitro. Recent reports have indicated that suppressor cells transferred to normal recipients must be homologous with the recipient at the Igh locus (24–26). Because the NP suppressor cells described above are idiotype specific, and because the gene that controls NP^b idiotype is linked to the $Igh\text{-}1^b$ locus (27, 28), it would be expected that these suppressor cells would fail to

TABLE II

*Effector-Phase Suppressor T Cells Specifically Adhere to NP^b Idiotype**

Cells added to culture	Fractionation of suppressor T cells	NP-specific PFC‡	
		Experiment 1	Experiment 2
5 × 10^5 control T	—	4,650	1,100
5 × 10^5 Ts	—	1,500 (68)	50 (95)
5 × 10^5 Ts	NP^b nonadherent	5,350 (−15)	1,700 (−55)
1–2 × 10^5 Ts	NP^b nonadherent	4,950 (−7)	1,250 (−14)
1–3 × 10^5 Ts	NP^b adherent	2,500 (46)	0 (100)
5 × 10^5 Ts	NP^j nonadherent	1,800 (61)	0 (100)
1–2 × 10^5 Ts	NP^j nonadherent	1,350 (71)	550 (50)
10^5 Ts	NP^j adherent	4,400 (5)	NT§

* C57BL/6 mice received an intravenous injection of 3 × 10^7 control or NP-coupled syngeneic spleen cells. 7 d later, these spleens were cultured for a 4-d subculture period. Control or suppressor cells from these subcultures were then T cell-enriched by passage over RAMIg-coated petri dishes before passage on NP^b- or NP^j-coated dishes. 5 × 10^5 viable control or fractionated T suppressor cells were then added to responder cultures challenged 4 d earlier with 100 ng NP-Ficoll. 1 d thereafter, duplicate wells were pooled and assayed for direct NP-specific PFC responses.

‡ The percent suppression relative to controls is presented in parentheses.

§ Insufficient numbers of cells were recovered from NP^j-coated plates to test this group.

TABLE III

*Igh Restriction of Effector-Phase Suppressor Cells In Vitro**

Responder cell donors	Cells added to culture	NP-specific PFC‡	
		Experiment 1	Experiment 2
CKB (Igh-1^b)	CKB control	5,150	2,830
	CKB suppressor	1,450 (72)	300 (89)
	C3H control	4,650	2,100
	C3H suppressor	4,680 (−1)	2,900 (−38)
C3H/HeJ (Igh-1^j)	CKB control	3,900	725
	CKB suppressor	4,250 (−9)	1,480 (−104)
	C3H control	3,580	1,430
	C3H suppressor	1,650 (54)	630 (56)

* CKB or C3H/HeJ mice received an intravenous injection of 3 × 10^7 control or NP-coupled syngeneic spleen cells. 7 d later, their spleen cells were cultured for a 4-d subculture period. 5 × 10^6 viable control or suppressor cells were then added to CKB or C3H/HeJ responder cultures that had been challenged 4 d earlier with 100 ng NP-Ficoll.

‡ The percent suppression relative to controls is indicated in parentheses.

suppress the response of a non-Igh-1^b strain. To test this prediction, spleen cells from Igh congenic CKB or C3H/HeJ mice that had been treated 7 d earlier with syngeneic control or NP-modified cells were subcultured for 4 d. 5 × 10^5 viable control or suppressor cells were then added to CKB (Igh-1^b) or C3H (Igh-1^j) responder cultures that had been challenged 4 d earlier with NP-Ficoll. 1 d later, cultures were assayed for NP-specific PFC responses. The data from two experiments are presented in Table III. Suppressor cells from both CKB and C3H mice were effective in reducing the response of syngeneic responder cells in the effector phase by 54–89%. However, neither suppressor population was capable of diminishing the response of Igh mismatched responders. Thus, CKB suppressor cells suppressed CKB but not C3H

responder cultures and vice versa. Effector-phase suppressor, therefore, must be homologous with the responder population at the Igh locus for expression of immune suppression.

Presence of Lyt-2 Determinants on Effector-Phase T Suppressor Cells. To further characterize the effector-phase suppressor cell, its Lyt phenotype was determined. Suppressor cells obtained from subcultures were treated with monoclonal, ABA-conjugated anti-Lyt-1 or anti-Lyt-2 antibodies, followed by a rabbit anti-ABA antiserum plus C. 1.8 or 3.5×10^5 viable control or treated suppressor cells were added to responder cultures challenged 4 d earlier. 1 d later, cultures were assayed for NP-specific PFC responses. The results from a representative experiment are presented in Table IV. Whereas treatment with anti-Lyt-1 antibody did not affect suppression, treatment with anti-Lyt-2 completely ablated the ability of suppressor populations to reduce an immune response in the effector phase. Thus, effector-phase T suppressor cells obtained after subculture of cells from hapten-modified spleen-cell-treated donors bear Lyt-2 determinants.

Specificity of Effector-Phase Suppressor Cells. It has previously been shown that antigen-induced suppressor cells may act nonspecifically in the effector mode once they have been stimulated specifically with antigen (29, 30). To determine whether suppressor cells obtained after subculture of cells from NP-spleen-cell-treated donors were capable of suppressing the response to an unrelated antigen in the effector phase, subcultured suppressor cells were added either to NP-KLH-primed responder cells challenged 4 d earlier with NP-Ficoll, normal responder cells primed 4 d earlier in vitro with SRBC, or 1:1 mixtures of NP-KLH-primed and normal cells challenged with NP-Ficoll and SRBC together. 1 d later, cultures were assayed for NP- and SRBC-specific PFC responses. The pooled and normalized data from five experiments are presented in Table V. Effector-phase, subcultured suppressor cells did not affect the ability of cultures of normal cells to respond to SRBC. In addition, in cultures in which both the NP- and SRBC-specific responses could be assayed simultaneously, subcultured cells that suppressed the NP-specific response 56% in the effector phase failed to significantly affect the SRBC-specific response. Thus, in these in vitro

TABLE IV
*Effector-Phase Suppressor Cells Bear Lyt-2 Determinants**

Cells added to culture	Antibody treatment	NP-specific PFC‡
3.5×10^5 control	—	3,100
3.5×10^5 suppressor	—	1,400 (55)
3.5×10^5 suppressor	Anti-Lyt-1 + C	1,300 (58)
3.5×10^5 suppressor	Anti-Lyt-2 + C	3,500 (−12)
1.8×10^5 suppressor	—	1,200 (61)
1.8×10^5 suppressor	Anti-Lyt-1 + C	1,700 (55)
1.8×10^5 suppressor	Anti-Lyt-2 + C	3,900 (−26)

* In this representative experiment, C57BL/6 mice received an intravenous injection of 3×10^7 control or NP-coupled syngeneic spleen cells. 7 d later, their spleens were subcultured for a 4-d period. Suppressor cells were then treated with ABA-conjugated anti-Lyt-1 or anti-Lyt-2 monoclonal antibody as indicated before treatment with rabbit anti-ABA plus C. 1.8×10^5–3.5×10^5 viable control or treated suppressor cells were added to responder cultures that had been challenged 4 d earlier with 100 ng NP-Ficoll. 1 d thereafter, duplicate wells were pooled and assayed for direct NP-specific PFC responses.

‡ The percent suppression relative to controls is indicated in parentheses.

TABLE V
*Specificity of Effector-Phase Suppressor Cells In Vitro**

Antigen	NP-specific PFC/culture $\overset{\times}{\div}$ log SE‡	SRBC-specific PFC/culture $\overset{\times}{\div}$ log SE	Percent suppression ± SE	
			NP-specific§	SRBC-specific
NP-Ficoll	1870 $\overset{\times}{\div}$ 1.4	—	70 ± 9	—
SRBC	—	1340 $\overset{\times}{\div}$ 1.7	—	8 ± 7
NP-Ficoll + SRBC	2390 $\overset{\times}{\div}$ 1.2	1530 $\overset{\times}{\div}$ 1.6	56 ± 13	15 ± 13

* In this pool of five experiments, C57BL/6 mice were given an intravenous injection of 3×10^7 control or NP-spleen cells. 7 d later, their spleens were subcultured for a 4-d period. 5×10^5 viable control or suppressor cells were then added to NP-KLH-primed responder cells challenged 4 d earlier with NP-Ficoll, normal responder cells primed 4 d earlier in vitro with SRBC, or 1:1 mixtures of NP-KLH-primed and normal cells challenged with NP-Ficoll and SRBC together. 1 d later, cultures were assayed for NP- and SRBC-specific PFC responses.

‡ Geometric mean $\overset{\times}{\div}$ log SE of PFC response per control culture.

§ Arithmetic mean ± SE.

experiments, effector-phase suppressors did not appear to be capable of nonspecifically affecting the primary response to an unrelated particulate antigen even when specifically stimulated by an ongoing, NP-specific immune response.

Discussion

In the present report, the ability of suppressor cells induced by the intravenous injection of NP-modified syngeneic spleen cells to affect the in vitro NP^b-idiotypic PFC response was studied. Previously, it had been shown that the intravenous injection of NP-modified syngeneic cells induced at least two subsets of T cells capable of suppressing delayed-type hypersensitivity (DTH) and cutaneous sensitivity responses in vivo (6, 23, 24, 26). One subset, termed induction-phase suppressor cells, bound to NP and was capable of specifically reducing a DTH response when given at the time of antigen priming. The second subset, referred to as effector-phase suppressor cells, adhered to NP^b idiotype and was shown to be capable of suppressing the DTH response when given 5 d after antigen priming, i.e., at the time of antigen challenge. Furthermore, it appeared that these subsets were causally related because injection of induction-phase suppressor cells with antigen induced effector-phase suppressor cells. An extension of this system demonstrated that spleen cells from mice given an intravenous injection of NP-conjugated syngeneic spleen cells were capable of suppressing the NP^b idiotype fraction of a PFC response either when transferred to normal syngeneic recipients or when subcultured for a 4-d period and then added to NP-KLH-primed responder cultures (17). In those initial in vitro experiments, subcultured suppressor cells were added to responder cultures at the time of antigenic challenge. Given the idiotype specificity of subcultured suppressor cells from NP-spleen-cell-treated donors, it appeared that these cells corresponded to the effector-phase, idiotype-specific DTH suppressor cells previously described (6). Based on this hypothesis, it was predicted that this population of cells would suppress an ongoing immune response in an idiotype-specific fashion. Therefore, in the present experiments, suppressor cells were added to in vitro cultures several days after antigenic challenge. The ability of these cells to suppress an ongoing immune response during

the efferent phase defined them as effector-phase suppressor cells. Because effector-phase suppressor cells were functional within a short period of time, it was likely that these cells represented a relatively mature population(s) of lymphoid cells. The maturational events required for differentiation to effector-phase suppressor cells appeared to take place during the 4-d subculture because spleen cells from donor mice treated 7 d earlier with NP-modified syngeneic cells were unable to suppress an in vitro response when added directly to responder cultures (data not shown). Furthermore, the ability of these cells to suppress an effector-phase response suggested that they correspond to second order suppressor cells (Ts_2 or Ts^e) described in other systems (1–3, 6).

The detection of the Lyt-2 determinant on effector-phase suppressor cells in the NP system was consistent with results obtained in other systems (31, 32). Thus, cells capable of effecting immune suppression in several systems were lysed by treatment with anti-Lyt-2 antiserum plus C but not by anti-Lyt 1 plus C (1, 26, 33). It is unlikely that this Lyt-2^+, T suppressor cell is a cytotoxic T lymphocyte because the NP-spleen cell-induced, effector-phase suppressor cell described in this (data not shown) and the related contact sensitivity system bears I-J region-encoded determinants that are thought to be absent on cytolytic T cells (26). Furthermore, the suppressor cells described in this report fail to kill NP^b-bearing hybridoma cell lines.

Effector-phase suppression appeared to be NP specific because subcultured NP-induced suppressor cells failed to alter the in vitro response to SRBC, even in the presence of the NP determinant. Evidence provided by both in vivo and in vitro studies suggested that suppression occurred at the B cell level. Thus, the response to NP-Ficoll, a relatively T-independent antigen (17, 34), was decreased by the addition of NP-induced suppressor cells in vitro. In addition, the magnitude of the NP-specific response to NP-poly-(L-Glu^{54}-L-Lys^{36}-L-Ala^{10}) in vivo was significantly decreased despite the presence of poly-(L-Glu^{54}-L-Lys^{36}-L-Ala^{10})-specific T helper cell activity (17).

The fine specificity of effector-phase suppression was determined by the measurement of suppression of idiotype-bearing B cell clones. For this purpose, a guinea pig antiserum was raised against purified anti-NP antibodies from C57BL/6 mice. This reagent was able to inhibit NP-specific plaque formation only in mice of the Igh-1^b allotype (17). With this reagent, it was possible to demonstrate that effector-phase suppressor T cells specifically affected the expression of idiotype-positive B cells either in vivo or in vitro. That is, idiotype-negative clones were not suppressed even in the face of active suppression of idiotype-positive cells. In fact, the suppression of NP^b-bearing clones in vitro appeared in some experiments to allow the expression of previously undetected NP^b negative B cells (Table I). This phenomenon may reflect the competition of NP^b idiotype-positive and -negative B cells for T helper activity or antigen required for terminal differentiation and expression of PFC. The ability of effector-phase suppressor cells to preferentially affect the NP^b idiotype-bearing B cell clones may have been brought about by one of two possible mechanisms. First, an antigen-bridging mechanism may have resulted in the selective suppression of cells that bore high affinity receptors for the hapten. If these high affinity cells corresponded to NP^b idiotype-bearing B cells, then these cells would have been selectively suppressed. Alternatively, immune suppression in the effector phase of a response may have been manifested by recognition of idiotypic determinants on target cells. Because

effector-phase suppressor cells bound idiotype-bearing antibody, it appeared that their interaction with target cells required recognition of idiotypic determinants. Thus, antigen bridging did not seem to be involved at least in the interaction of effector-phase suppressor cells and their immediate target lymphocytes.

The requirement for recognition of idiotypic determinants by effector-phase suppressor cells was further substantiated by their inability to function across an Igh allotype barrier. Thus, the absence of a homologous idiotype in Igh-mismatched recipients of effector-phase suppressor cells precluded recognition of target cells and thereby inhibited suppressor function. Similar findings have been reported in other systems in which interaction of T cell subsets was required for manifestation of immune suppression (4, 24–26). The ability of suppressor cells induced in C3H/HeJ mice to suppress the ongoing response of syngeneic responder cells in vitro suggested either that a relatively homogeneous suppressor population recognized a predominant idiotype (NP^j) common to all C3H mice or that the suppressor cell population was heterogeneous and recognized a series of idiotypes common only to that strain.

Although the effector-phase suppressor T cell described in this work recognized molecules bearing the NP^b idiotype determinant and the overall effect of the suppression was the specific inhibition of idiotype-bearing B cells, there is, to date, no direct evidence proving that the effector-phase suppressor cell was in fact the final effector cell in the suppressor pathway. Thus, it is possible that the Lyt-2^+ effector-phase T cell induced by subculture of spleen cells from NP-syngeneic cell-treated donors was merely an intermediary that interacted with and stimulated the final cell in the suppressor circuit whose direct target was the NP^b idiotype-bearing B cell. Results obtained in other systems have suggested that a "T auxiliary" or Ts_3 cell present in antigen primed populations was required for effector-phase (i.e., idiotype-specific) T suppressor cells to express their suppressive function (26, 35). Experiments designed to elucidate this suppressor pathway further are currently underway.

In this report we have described a system in which idiotype-specific, effector-phase suppressor T cells were induced with antigen-modified syngeneic spleen cells. This study departed in that regard from other investigations in which idiotype-reactive T cells were generated by injection of idiotypic or anti-idiotypic antibodies (10, 11, 15, 18–20). Although induction of T cell suppressor circuits with these antibodies is possible, induction of regulatory T cells with antigen probably reflects the physiologic role of these cells more accurately. The study of such a hapten-induced suppressor cell circuit will help clarify the nature of cellular interactions required to regulate the immune response to foreign antigens or to self antigens presented in the context of self cell surface determinants. It is important to emphasize that the effector-phase suppressor cells described in the present report have the same properties (idiotype binding, effector phase, Igh restricted, Lyt-2 bearing) as the effector-phase suppressor T cells, which suppress DTH and contact sensitivity responses (6, 24, 26). Thus, it appears that the same suppressor cell populations are able to regulate both T cell and B cell responses.

Summary

The ability of T suppressor cells, induced by the intravenous injection of 4-hydroxy-3-nitrophenyl acetyl (NP)-modified syngeneic spleen cells, to affect an ongoing B cell response was studied in vitro. It was found that the expression of NP^b idiotype-positive

B cells could be selectively inhibited by the addition of antigen-induced suppressor cells in the last 24 h of the in vitro culture. This effector-phase suppression of B cell responses was antigen specific and mediated by an Lyt 1^-, Lyt 2^+, idiotype-binding, T cell population whose suppressive function was restricted by genes linked to the Igh locus.

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