ANTIGEN RECEPTORS ON MURINE T LYMPHOCYTES IN CONTACT SENSITIVITY

III. Mechanism of Negative Feedback Regulation by Auto-Anti-Idiotypic Antibody*

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Regulation of the immune response by interactions between idiotypes (Id[1]) and anti-Id has been established in several different experimental systems (for review see 1–4). In some, administration of exogenous anti-Id antibody has been used, whereas in others the production of auto-anti-Id during the course of an immune response has been identified (5–10). In general, the appearance of auto-anti-Id correlates with the down-regulation of the immune response. The mechanisms involved in this down-regulation are unclear, although suppressor T cells (Ts) (10), inhibition of antibody secretion by anti-Id binding to B cell receptors (9), and elimination of Id$^+$ cells by complement (C)-mediated lysis (11) have all been implicated.

We have shown that the duration of the primary contact sensitivity (CS) response to 2,4-dinitrofluorobenzene (DNFB) is down-regulated by auto-anti-Id antibodies (12). These anti-Id antibodies, which are detected in suppressive immune serum (SIS) 9–15 d after DNFB sensitization, specifically block the transfer of CS to DNFB. In addition to this naturally occurring auto-anti-Id serum, we have also prepared a syngeneic anti-Id serum in BALB/c mice by repeated immunization with purified DNFB-sensitized lymph node (LN) T cells (T$_{DH}$-DNP) from BALB/c mice. The properties and specificity of this anti-Id serum, termed syngeneic anti-T$_{DH}$-DNP serum, are described elsewhere.[2] These studies have shown that the auto-anti-Id and the syngeneic anti-Id have essentially the same properties. During the characterization of the syngeneic antiserum, we noted that the transfer of CS to DNFB was inhibited by treating immune LN cells with the serum with or without C. The same results occurred when T$_{DH}$-DNP cells were treated with the auto-anti-Id serum. Reasoning

that anti-Id inhibition of transfer of immunity without C might be relevant to in vivo regulation by anti-Id, I have examined the phenomenon in some detail. The results show that this inhibition is an active process resulting from anti-Id activation of a subset of Ia^+ T cells in the immune LN cell population. The final suppression mediated by the anti-Id-activated T cells is antigen nonspecific.

Materials and Methods

Mice.   Male CBA/J mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

Sensitization with DNFB or Oxazolone (Ox).   To serve as donors of immune LN cells, mice were sensitized with DNFB by two daily paintings with 25 μl of 0.5% DNFB on the shaved abdomen and 5 μl on the footpads and ears. Ox sensitivity was induced by two paintings of 50 μl of 3% Ox on the shaved abdomen and 5 μl on the footpads and ears. In some experiments, donor mice were doubly sensitized with DNFB and Ox. This was done by painting the left side of the clipped abdomen, left forepaw, and ear with DNFB and the right side of the abdomen, right forepaw, and ear with Ox.

Transfer of Contact Sensitivity.   3 d after the last skin painting, single-cell suspensions of draining LN cells were prepared, and 4×10^7 to 5×10^7 cells were injected intravenously into normal syngeneic recipients. All recipient groups contained at least four animals. The recipients and negative controls were challenged within 1 h after cell transfer by applying 20 μl of 0.2% DNFB or 20 μl of 1% Ox on the dorsal side of each ear. Increased ear swelling was measured 24 h later with an engineer's micrometer and expressed in units of 10^{-4} in.

Antiserum.   Anti-Ia^k serum was prepared by repeated injections of A.TH mice with spleen and LN cells from A.TL donors. Polyvalent rabbit anti-mouse immunoglobulin serum (anti-MIg) was prepared as previously described (13). Before use, the antisera were heat inactivated at 56°C. Anti-MIg was also adsorbed with normal mouse thymocytes and erythrocytes (1:10 vol/vol). The cytotoxicity and specificity of both antisera have been described (13, 14).

Syngeneic anti-T_{DH}-DNP serum (anti-Id) was prepared by repeatedly immunizing BALB/c mice with purified LN T cells from DNFB-sensitized BALB/c mice.[2] SIS, which contains auto-anti-Id antibodies (12), was obtained by bleeding optimally DNFB-sensitized BALB/c mice 9, 12, and 15 d after sensitization, and pooling the serum.

For treatment of immune LN cells, 10^8 cells/ml were suspended in 1:10 diluted anti-Ia^k or anti-MIg, 1:2 diluted SIS, or 1:20 diluted syngeneic anti-T_{DH}-DNP serum, and incubated for 1 h on ice. After washing, anti-Ia-, SIS-, and anti-T_{DH}-DNP serum-treated cells were resuspended in 1:20 rabbit C (unless otherwise indicated), and anti-MIg-treated cells were resuspended in 1:6 guinea pig C. The cells were then incubated for 10 min on ice followed by 30 min at 37°C.

Purification of T Cells.   LN T cells were purified on nylon wool columns using a modification (15) of the procedure described by Julius et al. (16). Contamination of the nylon wool nonadherent population with B cells, as judged by immunofluorescent staining with fluorescein-labeled rabbit anti-MIg (Meloy Laboratories Inc., Springfield, VA), was ≤1%.

Preparation and Use of Immunoadsorbent Columns.   Affinity-purified normal MIg or BALB/c anti-DNP antibodies (MaDNP) were conjugated to Sepharose 4B beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) according to the standard labeling protocol. 20-ml plastic syringes filtered with plastic discs (Bel-Art Products, Pequannock, NJ) were packed with 5–7 ml of the appropriate Sepharose conjugate. Before use, the columns were washed with 60 ml phosphate-buffered saline (PBS, pH 7.3) and 30 ml 0.1 M NH_4OH, and then equilibrated with PBS. 2.5 ml of 1:20 syngeneic anti-T_{DH}-DNP serum was then added to the column and washed through slowly with 25 ml of PBS. 25 ml of effluent was collected and concentrated to 2 ml using an Amicon Ultrafiltration cell fitted with an XM50 Diaflo Ultramembrane (Amicon Corp., Scientific Sys. Div., Lexington, MA). Column-bound material was eluted with 25 ml of 0.1 M NH_4OH. The eluate was neutralized immediately with 1 N HCl, dialyzed against PBS and concentrated to 2 ml as described above.

Results

Inhibition by Anti-T_{DH}-DNP Serum of Transfer of Immunity: Lack of Requirement for C. As shown previously,[2] treatment of DNFB-immune LN cells with syngeneic anti-T_{DH}-DNP serum plus C inhibits the ability of the treated cells to transfer immunity to DNFB. We found, however, that C was not absolutely required for the inhibition of transfer of CS. Retesting of anti-Id-containing SIS gave similar results; SIS treatment of DNFB-immune LN cells inhibited transfer of sensitivity, whether or not C was added. Representative results of this basic phenomenon for the two sera are given in Table I. Experiment 1 shows results obtained using unseparated DNFB-sensitized LN cells, whereas results given for experiment 2 were obtained using nylon wool-purified DNFB-sensitized LN T Cells. As shown, similar levels of inhibition of transfer of immunity occurred whether or not the antiserum-treated cells were subsequently treated with C.

All experiments reported here have been done comparing SIS with anti-T_{DH}-DNP serum, and similar results have been obtained. For ease of presentation, however, only those results obtained using syngeneic anti-T_{DH}-DNP serum will be shown.

Inhibition of Transfer of Immunity by Treating LN Cells with Anti-T_{DH}-DNP Serum without C Requires Ia^+ T Cells in the Immune LN Population. At least three explanations would account for the inhibition of transfer of immunity by antiserum treatment without C. These are (*a*) anti-Id antibodies blocking the antigen receptor on T_{DH}-DNP effector T cells; (*b*) there is C-mediated lysis of the effector T cells after transfer to the normal recipients; and (*c*) anti-Id activates some T cell subset resulting in suppression of the effector T_{DH}-DNP cells. This latter possibility was considered because we have shown that LN cells from DNFB-sensitized mice are heterogeneous, containing at least three subsets of T cells, i.e., Ia^- T_{DH}-DNP effector T cells (14), Ia^+ T cells (Tprlf), which proliferate upon antigen restimulation in vitro (14), and Ia^+ auxiliary suppressor T cells (Ts-aux), which together with Ts suppress effector functions of the T_{DH}-DNP cells (17). To specifically look at the third possibility, LN cells from DNFB-sensitized mice were pretreated with NMS or anti-Ia serum plus C. The washed cells were then treated with syngeneic anti-T_{DH}-DNP serum plus or minus C and transferred to

TABLE I

Complement-independent Inhibition of Transfer of Contact Sensitivity to DNFB by Treatment with Anti-T_{DH}-DNP Serum or Suppressive Immune Serum

Experiment	Group	Serum treatment of immune LN cells	Experiment 1*		Experiment 2‡	
			Δ Ear swelling (× 10⁻⁴ in. ± SEM)	Percent inhibition	Δ Ear swelling (× 10⁻⁴ in. ± SEM)	Percent inhibition
I	A	NMS + C	42.8 ± 3.1		26.5 ± 1.9	
	B	Anti-T_{DH}-DNP + C	11.7 ± 1.9§	73	6.2 ± 2.1§	77
	C	Anti-T_{DH}-DNP	15.9 ± 2.1§	63	10.6 ± 1.4§	60
II	A	NMS + C	33.4 ± 3.6		24.0 ± 2.2	
	B	SIS + C	14.6 ± 0.8§	56	11.7 ± 1.4§	51
	C	SIS	16.4 ± 2.2§	51	10.1 ± 2.9§	58

* Unseparated LN cells used in experiment 1.
‡ Nylon wool-purified LN T cells used in experiment 2.
§ $P < 0.001$.

syngeneic recipients to assess their ability to transfer immunity. The results are given in Table II. Treatment of immune LN cells with NMS plus C did not alter the ability of the syngeneic anti-T_{DH}-DNP serum (without C) to inhibit the transfer of immunity (compare group B with A). In contrast, pretreating the immune LN cells with anti-Ia plus C eliminated the inhibitory effect of the syngeneic antiserum (group C). However, if the syngeneic antiserum was used with C (group D), transfer of immunity by the immune LN cells depleted of Ia^+ cells was inhibited. Depletion of B cells by pretreatment with anti-MIg plus C (group E) had no effect on the C-independent inhibition of transfer. These results indicate that the C-independent inhibition of transfer of immunity by syngeneic anti-T_{DH}-DNP serum requires the presence of Ia^+ T cells in the DNFB-sensitized LN population.

Ia^+ T Cells Needed for C-independent Inhibition by Syngeneic Anti-T_{DH}-DNP Serum Are Found in Sensitized but Not Normal LN Cells. To determine if the Ia^+ T cells were ubiquitous or required antigen activation, 2×10^8 DNFB-immune LN cells depleted of Ia^+ cells were mixed with untreated LN T cells from normal or DNFB-sensitized mice. The cell mixtures were then treated with syngeneic anti-T_{DH}-DNP serum without C and transferred to normal recipients that were then ear challenged with DNFB. Results of such an experiment are given in Table III. As previously described, immune LN cells depleted of Ia^+ cells were no longer inhibited by anti-T_{DH}-DNP serum without C (group C). Addition of 10^8 untreated LN T cells from DNFB-sensitized mice restored the inhibition (group D), whereas addition of 10^8 normal LN T cells had no effect (group E). These findings indicate that antigen activation is necessary to induce Ia^+ T cells, which are required for C-independent inhibition by syngeneic anti-T_{DH}-DNP serum of transfer of immunity.

Complement-independent Inhibition of Transfer of Immunity Induced by Syngeneic Anti-T_{DH}-DNP Serum is due to Anti-Id Antibodies. When used with C, the inhibitory activity of syngeneic anti-T_{DH}-DNP serum is due to anti-Id antibodies.[2] Thus, experiments were done to determine if anti-Id antibodies were also responsible for the C-independent inhibition of transfer of immunity. 2.5 ml of 1:20 syngeneic anti-T_{DH}-DNP serum were passed through Sepharose columns conjugated with either affinity-purified MIg or mouse anti-DNP antibodies. The column effluent and NH_4OH eluate were

TABLE II

Complement-independent Anti-T_{DH}-DNP Serum Inhibition of Transfer of Contact Sensitivity: Requirement of Ia^+ T Cells

Group	Serum treatment of DNFB-sensitized LN cells		Experiment 1		Experiment 2	
	1st treatment	2nd treatment	Δ Ear swelling ($\times 10^{-4}$ in. ± SEM)	Percent inhibition	Δ Ear swelling ($\times 10^{-4}$ in. ± SEM)	Percent inhibition
A	None	NMS + C	69.0 ± 5.7		42.8 ± 4.0	
B	NMS + C	Anti-T_{DH}-DNP	29.4 ± 3.1*	57	15.0 ± 1.9*	65
C	Anti-Ia + C	Anti-T_{DH}-DNP	71.6 ± 6.1	0	36.9 ± 3.3	14
D	Anti-Ia + C	Anti-T_{DH}-DNP + C	23.9 ± 2.2*	65	16.3 ± 0.8*	62
E	Anti-MIg + C	Anti-T_{DH}-DNP	31.7 ± 3.4*	54	15.9 ± 1.9*	63

* $P < 0.001$.

TABLE III

Ia^+ T Cells Required for Complement-independent Anti-T_{DH}-DNP Serum Inhibition of Transfer of Contact Sensitivity Are Induced by Antigen

| Group | Treatment of DNFB-sensitized LN cells | | | Δ Ear swelling ($\times$ 10^{-4} in. ± SEM) | Percent inhibition |
	1st treatment	Cells added after 1st treatment*	2nd treatment		
A	NMS + C	—	NMS	50.5 ± 4.8	
B	NMS + C	—	Anti-T_{DH}-DNP	19.2 ± 2.5‡	62
C	Anti-Ia + C	—	Anti-T_{DH}-DNP	49.6 ± 3.0	2
D	Anti-Ia + C	Untreated DNFB-sensitized LN T cells	Anti-T_{DH}-DNP	26.0 ± 3.1§	48
E	Anti-Ia + C	Normal LN T Cells	Anti-T_{DH}-DNP	56.2 ± 5.7	0

* LN T cells purified on nylon wool.
‡ $P < 0.001$.
§ $P < 0.01$.

TABLE IV

Anti-Id Antibodies in Anti-T_{DH}-DNP Serum Mediate the Complement-independent Inhibition of Transfer of Contact Sensitivity

| Group | Serum treatment of DNFB-sensitized LN cells | | Δ Ear swelling ($\times$ 10^{-4} in. ± SEM) | Percent inhibition |
	Serum	Treatment of serum		
A	NMS	—	33.8 ± 2.9	
B	Anti-T_{DH}-DNP	MIg column effluent	7.6 ± 1.7*	78
C	Anti-T_{DH}-DNP	MIg column eluate	35.5 ± 4.1	0
D	Anti-T_{DH}-DNP	MaDNP column effluent	30.4 ± 3.8	10
E	Anti-T_{DH}-DNP	MaDNP column eluate	3.0 ± 0.6*	91

* $P < 0.001$.

collected and concentrated, and tested without C on DNFB-immune LN cells for inhibition of transfer of immunity. The results are given in Table IV. Passage of the antiserum through the MIg column had no effect as all of the inhibitory activity was found in the column effluent (group B). In contrast, when the antiserum was passed through the MaDNP column, virtually all of the inhibitory activity was found in the column eluate (group E), indicating that the anti-Id antibodies in the syngeneic anti-T_{DH}-DNP serum are responsible for the C-independent inhibition of transfer of immunity.

Suppression by Anti-Id-activated Ia^+ T Cells is Antigen Nonspecific. In some experimental systems it has been shown that the final step of suppression in regulatory pathways is antigen nonspecific (18). To examine this question in the contact system, LN cells from mice doubly sensitized with DNFB and Ox were prepared and treated with either NMS or syngeneic anti-T_{DH}-DNP serum (10^8 cells/ml of 1:20 serum) for 1 h on ice. After washing, the cells were resuspended at 10^8 cells/ml and 0.5 ml injected i.v. into normal recipients. DNFB ear challenges were made on half of the recipients receiving NMS-treated cells, half receiving anti-T_{DH}-DNP serum-treated cells, and four negative control mice. The remaining recipients and negative controls were challenged with Ox and ear swelling was measured 24 h later. As shown in Table V, the transfer of CS to DNFB by LN cells from the doubly-sensitized mice was inhibited by treating the cells with syngeneic anti-T_{DH}-DNP serum (group B). However, the

TABLE V

Passive Transfer of Systemic Immunity: Complement-independent Inhibition by Anti-Id Antibody Appears Antigen Specific

Group	LN cells transferred ($\times 10^7$)	Serum treatment of LN cells from DNFB + Ox-sensitized mice	Ear challenge antigen	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	Percent inhibition
A	5.0	NMS	DNFB	28.9 ± 2.1	
B	5.0	Anti-T_{DH}-DNP	DNFB	5.2 ± 0.7*	82
C	5.0	NMS	Ox	36.9 ± 4.1	
D	5.0	Anti-T_{DH}-DNP	Ox	32.2 ± 3.6	13

* $P < 0.001$.

TABLE VI

Final Suppression Induced by Anti-Id Antibody is Antigen Nonspecific and Occurs Locally

Group	Cells transferred Intraear	Cells transferred Intravenous	Ear challenge antigen	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	Percent inhibition
A	NMS-treated DNFB-immune LN	Ox-immune LN	Ox	42.8 ± 3.7	
B	Anti-T_{DH}-DNP-treated DNFB-immune LN	Ox-immune LN	Ox	6.7 ± 2.3*	84

* $P < 0.001$.

ability of these same LN cells to transfer CS to Ox was unaffected by the antiserum treatment (group D). These results indicate that the inhibition is antigen specific. However, we considered the possibility that the suppressive signals delivered by the anti-Id-activated, Ia$^+$ T cells might occur locally at the skin test site. If so, anti-Id-treated DNP-specific T cells injected intravenously into mice challenged with Ox would not arrive at the skin test site to any significant degree. This could account for the results given in Table V. To explore this possibility, the following experiment was done. LN cells from DNFB-sensitized mice were treated in vitro with NMS or anti-T_{DH}-DNP serum. After washing, the cells were resuspended at 3×10^8 to 4×10^8 cells/ml in RPMI medium and 10 μl ($3-4 \times 10^6$ cells) injected intradermally into the dorsal side of the ears of normal mice. LN cells from Ox-sensitized mice were then prepared and 5×10^7 cells were injected intravenously into the mice previously given the intraear (IE) injection. Negative control mice received normal mouse serum (NMS)-treated DNFB-immune cells IE, but did not receive Ox-immune cells. All mice were ear challenged with Ox, and ear swelling was measured 24 h later. Results of such an experiment are given in Table VI. As shown, IE injection of anti-T_{DH}-DNP serum-treated LN cells from DNFB-sensitized mice suppressed the transfer of immunity by intravenous injected Ox-immune LN cells by 84%. This antigen-nonspecific suppression is dependent on Ia$^+$ cells in the DNFB-immune LN cell population as shown in Table VII. Here, the same protocol was used, except that the DNFB-sensitized LN cells were pretreated with anti-Ia serum plus C before treatment with the syngeneic anti-T_{DH}-DNP serum. Pretreatment with anti-Ia serum plus C reversed suppression of the Ox response from 83% (group B) to 28% (group C), which was not significantly different from the control response (group A).

TABLE VII

Local Antigen-Nonspecific Suppression Requires Ia^+ T Cells

| Group | Pretreatment of DNFB immune LN cells | Cells transferred | | Ear challenge antigen | Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM) | Percent inhibition |
		Intraear	Intravenous			
A	NMS + C	NMS-treated DNFB-immune LN	Ox-immune LN	Ox	38.1 $\pm$ 4.3	
B	NMS + C	Anti-T_{DH}-DNP-treated DNFB-immune LN	Ox-immune LN	Ox	6.5 $\pm$ 1.6*	83
C	Anti-Ia + C	Anti-T_{DH}-DNP-treated DNFB-immune LN	Ox-immune LN	Ox	27.3 $\pm$ 3.8	28

* $P < 0.001$.

Discussion

Considerable experimental evidence now exists supporting Jerne's proposal (19) that the magnitude and duration of an immune response is regulated, in part, by interactions between Id and anti-Id antibodies or membrane receptors. Of particular interest are those experimental systems which have shown, after immunization with antigen, the production of auto-anti-Id antibodies (5–12). These systems provide direct proof for the potent regulatory effects of anti-Id antibodies that occur under natural conditions. We have shown that CS to DNFB is regulated by auto-anti-Id antibody. Specifically, effector functions of the DNFB-sensitized T cells (T_{DH}-DNP) are inhibited by auto-anti-Id, which appears in the serum of mice 9–15 d after DNFB sensitization (12). The experiments described in this report were done to investigate the mechanism(s) involved in this regulation. Using transfer of CS to mimic the natural effector phase of the reaction and either auto-anti-Id or syngeneic anti-Id antibodies, the results indicate that in vivo regulation by anti-Id is a complement-independent, active process which involves anti-Id activation of a subset of Ia^+, Id^+ T cells. These T cells are present in the immune LN cell population but not in normal populations indicating that they are induced by antigen. Once activated by anti-Id, these T cells suppress effector mechanisms of the CS reaction in an antigen-nonspecific way.

Previous studies from our laboratory have shown the presence of functional Ia^+ T cells in LN populations from mice optimally sensitized with DNFB. Some of these T cells (Tprlf) proliferate in vitro upon restimulation with antigen (14). Of particular interest here, however, is another functional subset we have termed auxiliary suppressor T cells (Ts-aux). Ts-aux is a regulatory population. The cells are $I-J^+$ and precursors are sensitive to cyclophosphamide and are relatively short lived (18). In order for T_{DH}-DNP effector cells to be regulated by efferent acting Ts, there is an absolute requirement for Ts-aux in the immune population. In other words, the target for the exogenous Ts is Ts-aux, rather than the effector T_{DH} population. We believe that Ts-aux and the Id^+, Ia^+ T cells we have identified here belong to the same T cell subset. Further characterization is necessary, however, to show identity.

Numerous studies have shown the requirement for T-T interactions in both positive and negative regulation of immune responses. Thus, it was not surprising to find that an interaction between T cells, Ts-aux, and efferent acting Ts, was required for

suppression of effector T_{DH} cells. What was surprising, however, was that one subset was present in the immune population and apparently remained immunologically silent unless Ts, raised in other animals, were added. Because efferent acting Ts (or other functional Ts, for that matter) are not detected in optimally sensitized mice, the role of Ts-aux cells in natural regulation of contact sensitivity remained obscure. The results presented in this report, we believe, clarify this issue. It appears that after antigen (DNFB) stimulation, Id^+ clones of T_{DH} and Ts-aux cells are expanded (Fig. 1). The CS response mediated by T_{DH} cells peaks 6 d after sensitization. By day 9 after sensitization, the CS response is no longer detectable, and this down-regulation is caused by auto-anti-Id antibodies (12). These antibodies are produced, I believe, in response to the expanded numbers of Id^+ T_{DH} and/or Ts-aux cells. Regulation is affected by the anti-Id antibodies binding to Id receptors on the Ts-aux population. This apparently activates the cells, which then suppress the effector T_{DH} cells (Fig. 1). As shown by the data in Tables V and VI, this suppression is not systemic, but rather occurs locally at the skin test site and is antigen nonspecific. For reasons that are not clear, the anti-Id antibodies do not sterically block the receptors on the T_{DH} cells, although this binding occurs as evidenced by adsorption (12) and by C-mediated lysis of the cells in vitro. This indicates that lysis in vivo probably does not occur to any significant degree. Furthermore, it may be that steric inhibition of T_{DH} does not occur in vivo, because once the anti-Id antibodies bind and cross-link the Id receptors, rapid shedding occurs with subsequent receptor re-expression.

An important question, which cannot be answered by the present results, is whether cross-linking of Id receptors on Ts-aux by anti-Id antibodies is the sole activating signal, or whether other signals are also required. Clearly, the results in Table VI, where anti-Id-treated DNFB-sensitized LN cells were injected directly into the ears of mice, which then received Ox-immune LN cells intravenously and Ox ear challenge, indicate that exogenous DNFB as challenge antigen is not required to activate the cells. However, this does not preclude the possibility that interactions between Ts-aux and antigen-bearing or other cells contained in the locally transferred DNFB-sensitized population, might occur and be necessary for induction of the suppressive effect. It is
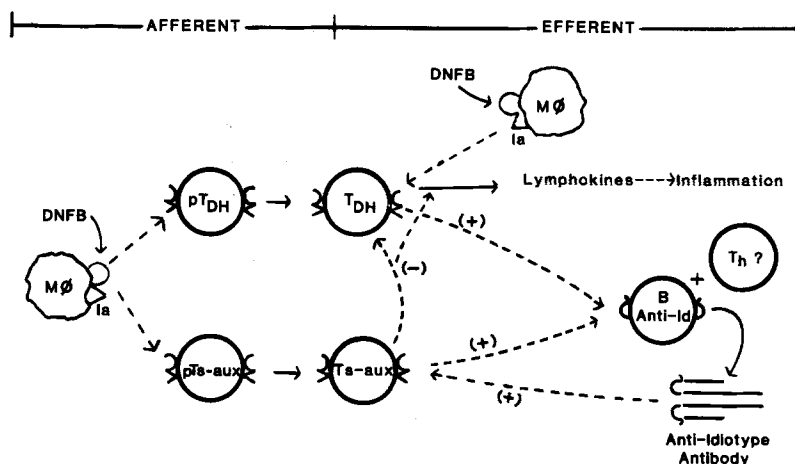

Fig. 1. Negative feedback regulation of contact sensitivity to DNFB by auto-anti-idiotype antibody.

likely that suppression is mediated by a soluble factor produced by Ts-aux, although we have no data in this regard. Experiments are currently underway to investigate these and other parameters of the system.

Recent data indicate that Ts-aux-like cells are present in other experimental systems and function to regulate immune responses. For example, in delayed hypersensitivity (DH) to p-azobenzenearsonate (ABA), Sy et al. (20) have recently identified a cross-reactive idiotype positive (CRI$^+$) T cell subset in ABA-sensitized LN cells that is required for anti-CRI, efferent-acting Ts to suppress the transfer of DH. Suppression occurred regardless of whether the T_{DH}-ABA effector cells were CRI$^+$ or CRI$^-$. Thomas et al. (21) have also identified a subset of T cells in ABA-sensitized lymphocytes that nonspecifically suppress transfer of DH following interaction with ABA-coupled spleen cells in vitro. This subset was shown to be CRI$^+$. Also, a recent report by Sugimura, et al. (22) suggests the presence of Ts-aux cells in phosphorylcholine (PC)-sensitized LN cells. They found that treatment of PC-sensitized T cells with anti-TI5 Id serum without complement blocked the transfer of PC-specific DH. Further characterization of other experimental systems is likely to reveal similar phenomena.

In conclusion, we have presented evidence indicating that natural regulation of CS to DNFB by auto-anti-Id antibodies involves a negative feedback regulatory loop. These data provide additional support for Jerne's network hypothesis (19) and further our understanding of the mechanisms involved in anti-Id regulation of T cell reactions.

Summary

Contact sensitivity (CS) to 2,4-dinitrofluorobenzene (DNFB) is maximal 6 d after sensitization but declines rapidly. Previous studies have shown that this rapid decline is due to auto-anti-idiotypic (anti-Id) antibodies produced by the host. The present study was done to investigate the mechanism(s) involved in this down-regulation of the effector phase of the CS reaction. Using transfer of CS to mimic the natural effector phase, we found that the inhibition of transfer by treating DNFB-sensitized lymph node (LN) cells with either auto-anti-Id or syngeneic anti-Id serum is complement (C) independent. This inhibition requires Ia$^+$ T cells in the immune population. Depleting immune LN cells of Ia$^+$ T cells rendered them insensitive to inhibition by anti-Id alone, although the same population is inhibited by anti-Id plus C. This cell population is rendered sensitive to inhibition by anti-Id alone by addition of untreated DNFB-sensitized LN cells, but not by addition of normal LN cells. Further studies showed that the suppression of immunity by anti-Id-activated Ia$^+$ T cells is not systemic, but rather occurs locally at the skin test site and is antigen nonspecific. We interpret these data to indicate that the natural regulation of CS to DNFB by auto-anti-Id antibodies is an active process that involves a negative feedback regulatory loop.

References

1. Binz, H., and H. Wigzell. 1977. Idiotypic, alloantigen-reactive T lymphocyte receptors and their use to induce specific transplantation tolerance. *Prog. Allergy.* **23**:154.

2. Rajewsky, K., and K. Eichmann. 1977. Antigen receptors of T helper cells. *Contemp. Top. Immunobiol.* **7**:69.

3. Bona, C. 1979. Regulation of lymphocyte function of anti-idiotypic antibodies. *In* Molecular Basis of Immune Cell Function. J. G. Kaplan, editor. Elsevier Scientific Publishing Co., Amsterdam. 161–180.

4. Bona, C., and J. Hiernaux. 1981. Immune response: idiotype anti-idiotype network. *Crit. Rev. Immunol.* **2**:33.

5. Kluskens, L., and H. Kohler. 1974. Regulation of the immune response by autogeneous antibody against receptor. *Proc. Natl. Acad. Sci. U. S. A.* **71**:5083.

6. Bankert, R. B., and D. Pressman. 1976. Receptor-blocking factor present in immune serum resembling autoanti-idiotype antibody. *J. Immunol.* **117**:457.

7. Cosenza, H., A. Augustin, and M. H. Julius. 1977. Induction and characterization of "autologous" anti-idiotypic antibodies. *Eur. J. Immunol.* **7**:273.

8. Kelsoe, G., and J. Cerny. 1979. Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature (Lond.).* **297**:333.

9. Schrater, A. E., E. A. Goidl, C. J. Thorbecke, and G. W. Siskind. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. I. Occurrence in AKR/J and BALB/c mice of hapten-augmentable, anti-TNP plaque-forming cells and their accelerated appearance in recipients of immune spleen cells. *J. Exp. Med.* **150**:138.

10. Gorczynski, R. M., M. Kennedy, B. Khomasurya, S. MacRae, and A. J. Cunningham. 1980. Individual-specific (idiotypic) T-B cell interactions regulating the production of anti-2,4,6-trinitrophenyl antibody. I. Generation of suppressor T cells and antibody against immunocompetent cells. *Eur. J. Immunol.* **10**:781.

11. Binz, H., and H. Wigzell, 1976. Specific transplantation tolerance induced by autoimmunization against the individual's own, naturally occurring idiotypic, antigen-binding receptors. *J. Exp. Med.* **144**:1438.

12. Sy, M-S., J. W. Moorhead, and H. N. Claman. 1979. Regulation of cell mediated immunity by antibodies: possible role of anti-receptor antibodies in the regulation of contact sensitivity to DNFB in mice. *J. Immunol.* **123**:2593.

13. Moorhead, J. W., C. S. Walters, and H. N. Claman. 1973. Immunologic reactions to haptens on autologous carriers. I. Participation of both thymus-derived and bone marrow-derived cells in the secondary *in vitro* response. *J. Exp. Med.* **137**:411.

14. Moorhead, J. W. 1978. Tolerance and contact sensitivity to DNFB in mice. VII. Identification of distinct T cell subpopulations that mediate *in vivo* and *in vitro* manifestations of delayed hypersensitivity. *J. Immunol.* **120**:137.

15. Moorhead, J. W. 1981. Antigen receptors on murine T lymphocytes in contact sensitivity. I. Functional inhibition of effector T cells by monovalent 2,4-dinitrophenol: implications for a two-receptor model. *J. Exp. Med.* **154**:1811.

16. 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.

17. Sy, M.-S., S. D. Miller, J. W. Moorhead, and H. N. Claman. 1979. Active suppression of 1-fluoro-2,4-dinitrobenzene-immune T cells: requirement of an auxiliary T cell induced by antigen. *J. Exp. Med.* **149**:1197.

18. Tada, T., and K. Okumura. 1979. The role of antigen-specific T cell factors in the immune response. *Adv. Immunol.* **28**:1.

19. Jerne, N. K. 1974. Towards a network theory of the immune response. *Ann. Immunol. (Paris).* **125C**:373.

20. Sy, M.-S., A. Nisonoff, R. N. Germain, B. Benacerraf, and M. I. Greene. 1981. Antigen-

and-receptor-driven regulatory mechanisms. VIII. Suppression of idiotype-negative, *p*-azobenzenearsonate-specific T cells results from the interaction of an anti-idiotypic second-order T suppressor cell with a cross-reactive-idiotype positive, *p*-azobenzenearsonate-primed T cell target. *J. Exp. Med.* **153**:1415.

21. Thomas, W. R., F. I. Smith, I. D. Walker, and J. F. A P. Miller. 1981. Contact sensitivity to azobenzenearsonate and its inhibition after interaction of sensitized cells with antigen-conjugated cells. *J. Exp. Med.* **153**:1124.

22. Sugimura, K., T. Kishimoto, K. Maeda, and Y. Yamamura. 1981. Demonstration of T15 idiotype-positive effector and suppressor T cells for phosphorylcholine-specific delayed-type hypersensitivity response in CBA/N or (CBA/N × BALB/c) F_1 male mice. *Eur. J. Immunol.* **11**:455.