EVIDENCE FOR SPECIFIC SUPPRESSION IN THE MAINTENANCE OF IMMUNOLOGIC TOLERANCE*

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The tolerant state to human gamma globulin (HGG)[1] in mice has been the most extensively studied model for tolerance to "self" in recent years (1). Indeed it is the only system in which the kinetics of the induction, maintenance, and loss of tolerance at the cellular level have been adequately defined (2–4). This tolerant state is of long duration and is stable upon adoptive transfer (1–4). The cellular kinetics of tolerance differ for thymus-derived lymphocytes (T cells) and bursal-equivalent-derived lymphocytes (B cells) and is antigen dose-dependent (2). The induction of this tolerant state can be blocked by the injection of bacterial endotoxin (5) or by aggregated HGG (6) only if given within a certain time before or after the injection of tolerogen. Tolerance to HGG in this system cannot be terminated by the injection of heterologous gamma globulins (7) nor by the transfer of normal lymphocytes (8) but can be terminated by the co-injection of HGG and bacterial endotoxin at a time when the B-cell compartment has recovered from tolerance (9).

The long duration of tolerance in the absence of further antigen contact could be due to an active phenomenon (i.e. suppression) rather than to a passive mechanism (i.e. simple absence of competent cells). Chiller and Weigle (8) were unable to demonstrate active suppression by HGG tolerant cells in an adoptive cell transfer system. However, Basten et. al. (10) have recently demonstrated the presence of specific suppressor cells in mice 6–10 days after the induction of tolerance to fowl gamma globulin. Active suppression has been demonstrated in a number of other systems (11–13). If the maintenance of the HGG tolerant state in mice is due to an active suppression mechanism then it is possible that: (*a*) the suppressor cells need time to exert their full effect; (*b*) in the absence of further antigen contact the frequency of these suppressor cells in a given population may decrease with time after the induction of tolerance; and (*c*) if the frequency of these cells does decrease with time, then the later the cells are taken after induction of tolerance the more time required for complete suppression. In

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[1] *Abbreviations used in this paper:* AHGG, ABGG, ASGG, aggregated HGG, BGG, SGG respectively; BGG, bovine γ-globulin; BSA, bovine serum albumin; BSS, Hanks' balanced salt solution; DNP-HGG, dinitrophenylated HGG; EGG, equine γ-globulin; FγG, fowl, γ-globulin; GRBC, goat red blood cells; HSA, human serum albumin; HGG, human γ-globulin; PFC, plaque-forming cells; PGG, porcine γ-globulin; PSA, porcine serum albumin; SGG, sheep γ-globulin; SSA, sheep serum albumin.

partial support of this are the observations of Herzenberg et. al. (11) which show that following co-transfer of normal spleen cells and spleen cells from chronically Ig-1b allotype suppressed mice, the quantity of Ig-1b allotype molecules in recipients increases for a brief period of time after which it declines to undetectable levels. These results suggest that a certain period of time was required for the suppressor cells to have their full effect on the co-transferred normal cells.

The studies described in this paper confirm and extend those of Ruben et. al. (7) on the cross-reactions of gamma globulins and the attempts to terminate the HGG tolerant state in mice. In addition, this paper also provides evidence for the existence of specific suppressor cells in mice tolerant to HGG at a time when the B-cell compartment has recovered immunocompetence to HGG but the T-cell compartment is still tolerant.

Materials and Methods

Animals. A/J male mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. Random-bred ICR male mice (of Swiss Webster lineage) were obtained from Flow Research Animals, Dublin, Va. All mice were maintained in groups of five to seven on Purina Chow Pellets (Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

Antigens. Bovine γ-globulin (BGG lot 46), porcine γ-globulin (PGG, lot 18), equine γ-globulin (EGG, lot 5), and sheep γ-globulin (SGG, lot 12) were obtained as Cohn fraction II from Miles Research Products, Elkhart, Ind. HGG was obtained as Cohn fraction II through the courtesy of the American Red Cross. All γ-globulins were further purified before use by elution from DEAE cellulose in 0.01 M phosphate buffer, pH 8.0. Deaggregated HGG or BGG (DHGG, DBGG) were prepared from the DEAE purified material by centrifugation at 140,000 *g* for 150 min in a Spinco model SW 50L rotor at 4°C (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) (6, 14). Aggregated γ-globulins (AHGG, ABGG, ASGG) were prepared by heating the DEAE purified material at 63°C and precipitation with Na_2SO_4 (6). Bovine serum albumin (BSA, lot J72104) was obtained from Armour Pharmaceutical Co., Kankakee, Ill. Human serum albumin (HSA, lot 10), porcine serum albumin (PSA, lot 10) and sheep serum albumin (SSA, lot 11) were obtained as Cohn fraction V from Miles Research Products, Elkhart, Ind. Goat erythrocytes (GRBC) were obtained in Alsever's solution from normal donors maintained at the University of Virginia. Dinitrophenylated HGG (DNP-HGG) was prepared as described by Eisen et. al. (15) and contained 40 moles DNP per mole HGG.

Labeling of Antigen. HGG was traced labeled with ^{125}I (New England Nuclear, Boston, Mass.) according to the method of McConahey and Dixon (16). For antigen carryover studies the HGG was labeled before centrifugation. Samples were counted in a gamma scintillation counter with a sodium iodide crystal.

Irradiation. Recipient mice were given 650 R whole-body irradiation. The mice were kept in a lucite chamber and placed in a Tele-Therapy unit with a 1.3 Mev, ^{60}Co source at a treatment distance of 80 cm and a dose rate of 91 R/min. These animals were reconstituted 4 h later. All irradiated mice were maintained on chlorinated-acidified water (17) to prevent early radiation death caused by Pseudomonas.

Immunizations. Mice were immunized with three intraperitoneal injections of 400 μg each of the appropriate γ-globulin or DNP_{40}-HGG, given 10 days apart as indicated in the text. Immunization with BSA was accomplished by giving 0.2 mg BSA emulsified in incomplete Freund's adjuvant subcutaneously in the scapular area, followed 3 wk later by a 1.0 mg booster given i.p. GRBC (5×10^8) were given i.p. followed by a booster injection (5×10^8) 10 days later.

Induction of Tolerance. 6-wk old mice were rendered tolerant by the intraperitoneal injection of 2.5 mg DHGG or DBGG in a 1.0 ml volume (6).

Cell Suspensions. All mice were sacrificed by cervical dislocation. Thymuses were removed from the thoracic cavity by careful dissection to avoid removal of the parathymic nodes. Spleens were removed from the peritoneal cavity and trimmed of fat. Single cell suspensions were prepared in

buffered salt solution (BSS) containing 100 μg streptomycin and 100 U penicillin per ml. The tissues were gently teased apart with forceps, pressing through stainless steel mesh and finally through a nylon sieve (18). The pooled suspensions were centrifuged at 250 *g* and washed 3–4 times in BSS before transfer. All procedures were carried out at 4°C.

Cell Transfers. The age and immunological status of all donors and recipients are as indicated in the text. Cell suspensions were injected intraperitoneally. Immunization with aggregated γ-globulins was begun immediately or beginning at 3 or 6 wk after cell transfer. 5 days after the last injection of immunogen the spleens were removed and assayed for antibody-forming cells.

Hemolytic Plaque Assay. PFC to GRBC, γ-globulins, and serum albumins were determined by a modification of the Jerne plaque assay (19, 20). Gamma globulins and serum albumins were coupled to indicator goat erythrocytes with a water soluble carbodiimide (Ott Chemical Co., Muskegon, Mich.) (20). All γ-globulins were absorbed with 1/10 volume of packed, washed GRBC at 4°C for 30 min before coupling. Indirect plaques were developed with goat antimouse γ-globulin at a concentration previously determined to be optimal in the assay.

Statistical Analyses. The Student's *t* test was used to determine the significance of the difference between means.

Results

Cross-reactions of Various γ-globulins in Mice. 8-wk old A/J male mice were each given three injections of 400 μg AHGG spaced 10 days apart. 5 days after the last injection these mice were sacrificed, and their spleens removed and assayed for plaque-forming cells to HGG and several other γ-globulins. It was seen that these mice produced little or no antibody that was cross-reactive with any of the other γ-globulins tested (Table I). Indeed the extent of cross-reaction as determined by the hemolytic plaque assay was 6% or less. To test whether this lack of cross-reactivity was due to the strain of mouse used, random-bred ICR mice were immunized with AHGG. Their spleens were assayed for PFC to HGG and to the other four γ-globulins. These mice were seen to produce a response 14 fold greater than did the A/J mice similarly immunized (Table I). In spite of this greater response, the degree of cross-reactivity was the same as seen in the A/J mice (cross-reaction of 9% or less, Table I). To determine whether this lack of cross-reactivity was peculiar to HGG the following experiments were performed.

TABLE I

Cross-reactions of Various Serum Proteins in Mice

Group	No. and strain of mice	Immunogen	Indirect PFC/10^6 spleen cells				
			BGG*	EGG	HGG	PGG	SGG
1	7 A/J	HGG	5 (3)‡	0	170 (100)	11 (6)	0
2	6 ICR	HGG	206 (9)	37 (2)	2,410 (100)	194 (8)	26 (1)
3	6 A/J	BGG	247 (100)	0	17 (7)	13 (5)	45 (18)
			Indirect PFC/10^6 spleen cells				
			BSA*	HSA	PSA	SSA	
4	5 A/J	BSA	360 (100)	10 (3)	27 (8)	156 (43)	

* Antigen coupled to GRBC for plaque assay.

‡ Mean of five to seven mice per group individually assayed; % cross-reaction in parentheses.

A/J mice were immunized with ABGG and their spleens assayed for PFC to BGG and other γ-globulins. In addition, other A/J mice were immunized to bovine serum albumin (BSA) and their spleens assayed for PFC to BSA and several other albumins. As with the A/J mice immune to HGG those immunized with BGG produced little or no cross-reacting antibody (Table I). In contrast, those mice immunized with BSA showed considerable crossreactivity with other albumins (Table I).

Response of Tolerant Mice to Injections of Crossreacting or Altered γ-globulins. Groups of normal mice and mice tolerant to HGG were given three injections of 400 μg AHGG, ABGG, or DNP_{40}-HGG. 5 days after the last injection, these mice were sacrificed and their spleens assayed for PFC to HGG and to the immunizing antigen. Normal mice immunized with AHGG or DNP_{40}-HGG produced antibody reactive with HGG (Table II). In contrast, HGG tolerant mice did not respond to any of the injections. In addition, the response of these mice to the specific determinants of BGG was reduced to 19% of that of normal mice immunized with BGG (compare Groups 4 and 9, Table II). To test whether the inability of cross-reacting γ-globulins to terminate tolerance to HGG was due to the strain of mice used, random-bred ICR mice were made tolerant to HGG, rested 80 days, and then immunized with AHGG or ABGG. As with the tolerant A/J mice above, these ICR tolerant mice did not respond either to injections of AHGG or ABGG (Table II). Similarly, mice tolerant to BGG were immunized

TABLE II

Capacity of Injections of Various Cross-reacting Gamma Globulins to Terminate Tolerance to HGG or BGG in Mice

Group	No. and strain of mice	Tolerogen	Immunogen	Indirect PFC					
				PFC/10^6			PFC/Spleen		
				BGG*	HGG	SGG	BGG	HGG	SGG
1	6 A/J	—	HGG	5‡	170	0	695	24,519	0
2	5 A/J	—	DNP-HGG§	—	69	—	—	10,574	—
3	14 A/J	HGG‖	HGG	—	7	—	—	730	—
4	7 A/J	HGG	BGG	48	0	—	6,762	0	—
5	6 A/J	HGG	DNP-HGG	—	0	—	—	0	—
6	7 ICR	—	HGG	—	2,410	—	—	285,773	—
7	4 ICR	HGG	HGG	—	13	—	—	2,525	—
8	9 ICR	HGG	BGG	—	6	—	—	944	—
9	7 A/J	—	BGG	247	17	45	34,062	2,262	6,053
10	7 A/J	BGG	BGG	17	—	—	2,076	—	—
11	6 A/J	BGG	SGG	2	1	1	83	—	33
12	6 A/J	BGG	HGG	1	1	—	33	83	—

* Antigen coupled to goat red blood cells for plaque assay.

‡ Mean of 4–14 mice per group individually assayed.

§ DNP-HGG: dinitrophenylated human γ-globulin; DNP_{40}-HGG.

‖ Includes mice immunized at 35, 56, and 77 days after the injection of tolerogen.

with ABGG, ASGG, or AHGG. These BGG tolerant mice also failed to respond to any of these injections (Table II). Again the response to specific determinants on the cross-reacting γ-globulin was severely reduced, i.e., the response to specific HGG determinants of BGG tolerant mice immunized with AHGG was reduced to less than 1% of that of normal mice similarly immunized (compare Groups 1 and 12, Table II).

Capacity of Lymphocytes from Normal or Immune Mice to Terminate Tolerance to HGG in Mice. A/J mice tolerant to HGG (56 days after the injection of tolerogen) were injected with 50 million normal syngeneic thymocytes, 100 million normal spleen cells, or 100 million immune spleen cells. These tolerant recipients were then given three injections of 400 μg AHGG spaced 10 days apart. Their spleens were removed 5 days after the last injection and assayed for PFC to HGG. As shown in Table III, none of these lymphocyte preparations were successful in reconstituting the tolerant mice for a response to immunogenic HGG. To determine whether the tolerant state could be transferred to normal, nonirradiated mice by tolerant spleen cells, the following experiment was performed. Spleens from tolerant A/J mice were removed 35 days after the induction of tolerance. Single cell suspensions were made and 100 million tolerant spleen cells were injected i.p. into normal 8-wk old A/J recipients. These recipients were then immunized as above and their spleens assayed for PFC to HGG. These normal recipients of tolerant cells did not respond to immunogenic HGG (Group 6, Table III) suggesting that tolerance was "infectious". In addition, preliminary data shows that 35 day tolerant mice, given 650 R whole-body irradiation, reconstituted with 100 million normal spleen cells and immunized with AHGG do not respond.

Adoptive Cell Transfer Studies. To test whether spleen cells from HGG tolerant mice could suppress the response of normal spleen cells, the following

TABLE III

Capacity of Spleen or Thymus Cells From Normal or Immune Mice to Terminate Tolerance to HGG in Mice

Group	Recipient status	No. and type of cells transferred	Indirect PFC	
			PFC/10^6	PFC/spleen
1	N*	0	170 ± 52‡	24,519
2	T	0	7 ± 3§	730
3	T	5×10^7 NT‖	9 ± 3§	1,881
4	T	10^8 NS	10 ± 3§	1,036
5	T	10^8 IS¶	2 ± 1§	171
6	N	10^8 TS	6 ± 1§	738

* N, normal; T, tolerant.

‡ Mean ± SE of 6–14 mice per group individually assayed.

§ $0.01 > P > 0.002$ relative to Group I.

‖ NT, normal thymus; NS, normal spleen; TS, tolerant spleen; IS, immune spleen.

¶ From normal donors previously immunized with 3×400 μg AHGG and spleens removed 6 wk later.

adoptive cell transfer studies were carried out. Groups of 8-wk old A/J mice were give 650 R whole-body irradiation and supplemented with normal spleen cells, or tolerant spleen cells plus normal spleen cells as indicated in Fig. 1 and Table IV. Normal donors were 8 wk of age and tolerant donors were used at 35, 56, or 77 days after the induction of tolerance. Each of these recipients were then given three injections of AHGG i.p. beginning 0, 3, or 6 wk after cell transfer. Mice that received normal spleen cells showed an increasing response with time after cell transfer (Groups 1–3, Table IV). In contrast, mice that received normal spleen cells plus 35 day tolerant spleen cells produced a significantly lower response (Groups 4–6, Table IV). Mice that received normal spleen cells plus 56 day tolerant spleen cells also produced little antibody relative to those receiving normal cells alone (Groups 7–9, Table IV). However, recipients of normal spleen cells plus 77 day tolerant spleen cells produced a normal response if challenged immediately after transfer (Groups 10, Table IV), but this response waned when immunization was delayed for 3 or 6 wk after transfer (Groups 11 and 12, Table IV). The extent of this suppression and its dependence on time for its full expression is shown diagramatically in Fig. 2.

Antigen Carryover. The results above could be explained by carryover of tolerogenic HGG with the tolerant spleen cells suspensions. To eliminate this possibility HGG was labeled with iodine-125 and the monomeric form prepared by ultracentrifugation at 140,000 *g* for 150 min. The top ⅓ of the centrifuged

TABLE IV

Capacity of Spleen Cells From HGG Tolerant Mice to Suppress the Immune Response of Normal Spleen Cells in an Adoptive Transfer System

Group	Source of cells*	Time of‡ challenge	Indirect PFC§	
			PFC/10^6	PFC/spleen
		wk		
1	NS	0	121 ± 29	7,033
2	NS	3	415 ± 92	19,906
3	NS	6	524 ± 114	32,857
4	NS + 35 day TS	0	6 ± 3 (<0.001)	350
5	NS + 35 day TS	3	29 ± 16 (<0.001)	2,828
6	NS + 35 day TS	6	38 ± 16 (<0.001)	3,022
7	NS + 56 day TS	0	38 ± 12 (=0.01)	2,672
8	NS + 56 day TS	3	20 ± 11 (<0.01)	834
9	NS + 56 day TS	6	20 ± 15 (=0.001)	1,408
10	NS + 77 day TS	0	148 ± 76 (>0.1)	8,238
11	NS + 77 day TS	3	71 ± 32 (=0.002)	2,836
12	NS + 77 day TS	6	20 ± 19 (<0.001)	963

* NS, normal spleen cells; TS, tolerant spleen cells; tolerant spleen cells were removed from donors 35, 56 or 77 days after the induction of tolerance. Each recipient received either 10^8 normal spleen cells or 10^8 normal spleen cells plus 10^8 tolerant spleen cells.

‡ Time after adoptive cell transfer that immunization of the recipient with aggregated HGG was begun.

§ Mean ± SE of the mean of 5–7 mice per group individually assayed. *P* values relative to appropriate control groups 1–3 given in parentheses.

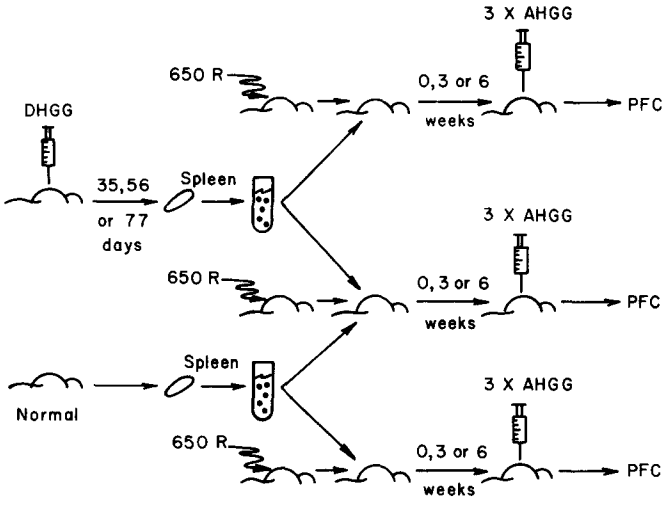

FIG. 1. Protocol for adoptive cell transfer studies.

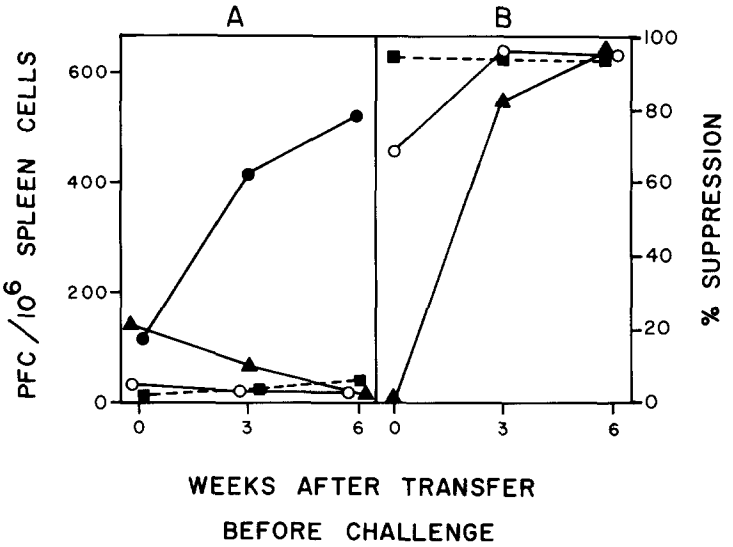

FIG. 2. Plaque-forming response and extent of suppression in mice receiving normal and tolerant spleen cells. (A) Plaque-forming cell response of mice to HGG after irradiation and supplementation with either normal spleen cells or normal spleen cells plus tolerant spleen cells; (B) Extent of suppression of the response of normal spleen cells by spleen cells from tolerant mice; recipients of normal spleen cells alone (●—●); recipients of normal spleen cells plus spleen cells taken from tolerant mice 35 days after the induction of tolerance (■---■); recipients of normal spleen cells plus spleen cells taken from tolerant mice 56 days after the induction of tolerance (O—O); recipients of normal spleen cells plus spleen cells taken from tolerant mice 77 days after the induction of tolerance (▲—▲).

solution was carefully removed and 2.5 mg [^{125}I]HGG (30,000 cpm/μg) was injected i.p. into 6-wk old A/J mice. 35 days later these mice were sacrificed, their spleens removed, and single cell suspensions made as above in the adoptive cell transfer studies. These spleen cells (100 million) were then counted and the

amount of HGG determined from the ^{125}I activity. This amount was determined to be less than 0.01 μg per 100 million spleen cells. This amount is several logs lower than that necessary to induce minimal tolerance in intact mice (1).

Specificity of Suppression. To determine whether this suppression was specific for HGG, the following studies were carried out. 8-wk old A/J mice were given 650 R whole-body irradiation followed by injections of either 10^8 normal spleen cells or 10^8 normal spleen cells plus 10^8 35 day tolerant spleen cells. These recipients were then challenged with AHGG as above or with GRBC as a specificity control. Mice that received tolerant plus normal cells again did not respond to injections of AHGG as compared to recipients of normal cells alone (Table V). In contrast, similar recipients did respond normally to injections of GRBC (Table V).

TABLE V

Specificity of Suppression by Spleen Cells From Mice Tolerant to HGG

Group	Source of cells*	Immunogen	PFC/10^6		PFC/spleen	
			Direct	Indirect	Direct	Indirect
1	10^8 N	HGG	ND	197‡	ND	21,334
2	10^8 N + 10^8 T	HGG	ND	12	ND	1,560
3	10^8 N	GRBC	67	208	2,940	9,450
4	10^8 N + 10^8 T	GRBC	166	340	10,173	26,600

* N, normal spleen cells; T, tolerant spleen cells taken from mice 35 days after the induction of tolerance.

‡ Mean of 5–7 mice per group individually assayed.

Discussion

The experiments reported here have demonstrated the presence of specific suppressor cells in mice tolerant to the thymus-dependent antigen HGG. Adult tolerance to HGG was induced by a single injection of monomeric HGG at a concentration sufficient to result in tolerance in both the B-cell and T-cell populations (1). The transfer of normal thymocytes, normal spleen cells, or immune spleen cells into these tolerant mice did not restore the capacity to respond to immunogenic HGG (Table III). In addition, the injection of tolerant spleen cells into normal intact mice abrogated the response of these normal recipients to immunogenic HGG (Table III).

Direct demonstration of suppressor cells in these HGG tolerant mice was accomplished by adoptive cell transfer studies. Spleen cells removed from mice 5, 8, or 11 wk after the induction of tolerance, significantly suppressed the response of normal spleen cells when co-transferred into lethally irradiated recipients (Table IV). The specificity of this suppression was shown by the ability of similarly treated mice to respond to injections of another, noncross-reacting, thymus-dependent antigen GRBC (Table V). The presence of residual tolerogen as the cause of suppression was eliminated in that, using radiolabeled HGG, no residual tolerogen could be found in the transferred spleen cells. Indeed as little

as 0.01 μg HGG could have been detected; an amount 1,000-fold less than that necessary to induce minimal tolerance in intact adult mice (1).

Several other studies lend support to the above observations. McCullagh was able to terminate tolerance to sheep erythrocytes in rats with allogeneic cells (21) but not with syngeneic spleen cells (22, 23). Chiller and Weigle (8) were unable to restore competence after transfer of normal thymocytes or spleen cells into mice tolerant to HGG. They could not, however, demonstrate suppression in adoptive transfer studies. Successful transfer of competence into tolerant recipients was accomplished in several systems (24, 25). Neonatally-induced tolerance to BSA in rabbits was terminated by transfer of normal sibling thymocytes (25) and could not be attributed to the allogeneic effect since the transfer of thymocytes from tolerant siblings did not result in termination.

Evidence for suppressor activity has been presented in studies from a number of laboratories (reviewed in ref. 13). More recently Basten et. al. (10) have demonstrated the existence of theta-bearing specific suppressor cells in mice tolerant to fowl γ-globulin (FγG) at the T-cell level only. These cells were found in spleen 6–10 days after the injection of tolerogenic FγG indicating the early appearance of suppressor activity. Furthermore, in agreement with our data, they showed that transfer of spleen cells from these tolerant mice into normal recipients specifically abrogated the response of these recipients to immunogenic FγG.

The extent of suppression, in the adoptive transfer studies reported here, appears to depend upon how long after the induction of tolerance the cells were removed from the tolerant donors and how soon after transfer the recipients were challenged with immunogenic HGG (Table IV, Fig. 2). Spleen cells removed from mice 5 wk after the induction of tolerance completely suppressed the response of normal cells whether challenged immediately after transfer or as long as 6 wk later. In contrast, suppression by cells removed 8 or 11 wk after the induction of tolerance was more complete if immunization of the recipients was delayed for several weeks (Table IV, Fig. 2). These results suggest that the frequency of, or the activity of, suppressor cells declines with time after the induction of tolerance, and that given enough time relatively fewer numbers of suppressor cells can exert a complete suppressive effect. These results also demonstrate the presence of suppressor activity at a time after the B-cell population has recovered from tolerance (1), suggesting that if the B cell is the immediate target of suppression then the mechanism of suppression is not cytolytic. However the target of suppressor activity is not known. The results presented here and in other reports (10, 13) would be just as compatible with specific helper T cells as the target since immunocompetence in the T-cell population does not return until considerably later than the return of B-cell competence. The results of this study, along with those of Basten et. al. (10), would suggest the early appearance of suppressor cells after the induction of tolerance and a gradual decline in the frequency of these cells (in the absence of further contact with tolerogen) resulting in an eventual return to competence.

In contrast to tolerance to serum albumins in rabbits (26, 27) tolerance to HGG in mice cannot be terminated by the injection of cross-reacting or altered gamma

globulins (Table II, ref. 7). That this is true for γ-globulins in general, and not peculiar to HGG or the strain of mice used, is shown by the inability of cross-reacting γ-globulins to terminate tolerance to BGG in A/J mice and to the inability of cross-reacting γ-globulins to terminate tolerance to HGG in ICR mice (Table II). Furthermore, these tolerant mice produce considerably less antibody to specific determinants on the cross-reacting γ-globulin (Table II, ref. 7) than that produced by normal mice similarly immunized. This lack of response to specific determinants has been attributed to extensive cross-reaction at the T-cell level (7). The demonstration of suppressor activity, as shown in this paper, suggests that a mechanism of cross-suppression similar to that postulated by Scibienski et. al. (28) may be operative, i.e. activation of suppressor cells in HGG tolerant mice by crossreacting determinants on the heterologous γ-globulin would result in suppression of the response to all determinants carried by the heterologous γ-globulin (both specific and cross-reacting). If true, one would predict that spleen cells from HGG tolerant mice would suppress the response of normal spleen cells to challenge with BGG in an adoptive transfer system.

As mentioned above, the target of the suppressor cells is not known. Specific elimination or inactivation of either or both of the specific cell types (T helper cells or B cells) required for a response to thymus-dependent antigens would result in a suppressed response. The presence of suppressor cells at a time after B cells have recovered from tolerance would suggest that suppression can occur at the T-cell level and that the maintenance of tolerance, in the absence of further antigen contact, is due to the inactivation of specific helper cells. Furthermore, the subsequent decline in frequency of these suppressor cells would eventually permit a reappearance of competent T cells and a restoration of full immunological capacity. If true, one would predict that continued contact with tolerogen (at doses less than that necessary to reinduce tolerance in B cells) would result in prolongation of suppressor activity resulting in an extension of the duration of tolerance. The effect of continued contact with tolerogen on suppressor activity is currently being studied.

Summary

Specific suppressor cells have been demonstrated in mice tolerant to the thymus-dependent antigen HGG. Transfer of normal thymocytes, normal spleen cells, or immune spleen cells into these tolerant mice did not restore immunocompetence to HGG. Furthermore, the transfer of tolerant spleen cells into normal recipients abrogated the response of these recipients to subsequent challenge with immunogenic HGG.

Spleen cells removed from mice 5, 8, or 11 wk after the induction of tolerance specifically suppressed the response of normal spleen cells in an adoptive cell transfer system. The extent of suppression appears to be dependent upon how long after the induction of tolerance the cells were removed from the tolerant donors and how soon after transfer the recipients were challenged.

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