ANTIGEN MEDIATION OF A LATE-ACTING SUPPRESSOR T-CELL ACTIVITY*

By ROBERT W. WARREN‡ and JOSEPH M. DAVIE

(From the Department of Microbiology and Immunology, and the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110)

Regulation of the avidity of an antibody response by T lymphocytes may require the ability of the T cell or its product to distinguish between cells which presumably differ only in the avidity of the immunoglobulin they secrete. The most likely means by which this difference could be expressed at the level of the B cell would be by the varying capacities of B cells to bind antigens to their surfaces. These antigen molecules in turn could be specific targets for T cells or their products.

Elsewhere (1), we describe a suppressor T-cell activity which inhibits high avidity IgG antibody secretion within 90 min. This suppression is mediated by carrier-specific T cells. Expression of the activity appears independent of helper T cell and macrophage function; it most likely involves direct action on the hapten-specific antibody-secreting cell itself. We demonstrate here that hapten carrier on the surface of high avidity plaque-forming cells (PFC)[1] most likely provides the necessary target for the suppressor cell. This implies that some specific B lymphocytes normally bear immunogen on their surfaces for at least several days. This bound antigen could be important to the maintenance and regulation of the normal immune response.

Materials and Methods

Animals. AKR/Nat mice were obtained from the National Laboratories, Creve Coeur, Mo.

Antigens and Immunizations. Keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, Calif.) and ovalbumin (EA) (Miles Laboratories Inc., Elkhart, Ind.) were conjugated with 2,4-dinitrofluorobenzene (DNP). DNP/KLH ratio was 7.4 groups/100,000 daltons; DNP/EA ratio was 19 groups/molecule. Conjugation ratios were determined from absorbance at 360 nm at a known protein concentration. 5- to 8-wk-old female mice were immunized by i.m. injections of 100 μg of hapten protein emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.). A secondary response was induced by the i.p. administration of 100 μg of antigen in saline at least 4 wk after primary immunization. Five mice comprised each experimental group.

Antisera. Goat anti-mouse μ-serum was described previously (1); rabbit anti-mouse IgG was purchased from Miles Laboratories Inc., and Gateway Immunosera (Cahokia, Ill.). Normal and

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[1] *Abbreviations used in this paper:* ATC, activated T cells; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrofluorobenzene; EA, ovalbumin; FCS fetal calf serum; Fl-anti-Ig, fluorescein-labeled rabbit anti-mouse Ig; KLH, keyhole limpet hemocyanin; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TNP-SRBC, 2,4,6-trinitrophenyl-coated sheep erythrocytes.

anti-KLH hyperimmune guinea pig sera were the gifts of Dr. Joyce Schroer (Washington University, St. Louis Mo.). These sera were heat inactivated at 56°C for 40 min; Ig fractions were then obtained by 40, and then 50% ammonium sulfate precipitations. After extensive dialysis against phosphate-buffered saline (PBS), these preparations were absorbed for 1 h at 4°C with equal volumes of both packed sheep erythrocytes (SRBC) and normal AKR mouse spleen cells.

Activated T Lymphocytes. AKR/Nat mice (8- to 12-wk-old) were lethally irradiated (750 R) and received 10^8 syngeneic thymocytes i.v. from 5-wk-old donors the same day. 100 μg of antigen (hapten-carrier conjugate or carrier alone) in saline was administered i.p. 7 days later, and spleens were removed and teased, yielding $\cong 10^7$ cells per spleen. Viability of this population ranged from 15 to 30%. It has been previously shown (1) that the suppressor activity in this cell population depends on Thy-1 bearing, nylon nonadherent T cells. Whether activated against DNP-carrier protein or carrier alone, such activated T cells (ATC) do not demonstrate significant direct or indirect DNP-specific PFC (<20 direct PFC and <1 indirect PFC/10^6 viable cells).

Suppressor Cell Assay. Spleen cells (8–10 × 10^6) of DNP-KLH immune mice were mixed with 10^7 antigen-primed ATC and incubated at 37°C for 90 min in 1.0 ml of minimal essential medium, Hanks' base, containing 10% fetal calf serum (FCS). In some experiments, ATC and/or hyperimmune spleen cells were pretreated with antigen or antibody preparations (see Results). After this incubation, the suspension was diluted appropriately for counting ≅200–300 PFC per slide.

Plaque Assay. Antibody-secreting splenocytes (PFC) were assayed by a modification of the localized hemolysis technique in agarose with 2,4,6-trinitrophenyl-coated sheep erythrocytes (TNP-SRBC) (2). Indirect PFC were quantitated by inhibition of >95% of direct PFC with goat anti-mouse μ-serum (at 1:4,000 final dilution) incorporated into the slide gel, and facilitation of indirect PFC with rabbit anti-mouse IgG at 1:200 dilution.

The plaque assay technique was further modified to delineate the avidity of secreted antibody by a hapten inhibition method (1). ϵ-DNP-L-lysine was incorporated in the gel in five molar concentrations: 10^{-4}–10^{-8} M; in some experiments, 10^{-4} M was omitted. An avidity subgroup was defined as those PFC present at a given inhibitor concentration, but absent at the next higher concentration. Thus, six avidity subgroups are created, named as the negative log of the molar concentrations of the inhibitor: <8 (highest avidity), 7–8, 6–7, 5–6, 4–5, >4 (lowest avidity).

Cell counts and viabilities were determined on a 4800A Cytofluorograf (Bio/Physics Systems Inc., Mahopac, N. Y.).

Pronase Treatment of Lymphoid Populations. Approximately 80 × 10^6 hyperimmune splenocytes, previously maintained in a protein-free medium, were incubated with 5.0 mg pronase CB (Calbiochem, San Diego, Calif.) and 100 μg DNase 1 (Worthington Biochemical Corp., Freehold, N. J.) in a total volume of 1.0 ml for 15 min at 37°C. Immediately thereafter, 2.0 ml FCS was added and the suspension centrifuged. After washing, the cells were maintained in a 10% FCS medium. The Ig-bearing subpopulation of hyperimmune cells before and after pronase treatment was quantitated using a fluorescein-labeled rabbit anti-mouse Ig (Fl-antigen-Ig) (3). Normal splenocytes contained 47% Ig-bearing cells; after pronase treatment, only 7% possessed detectable Ig. After pronase treatment, the hyperimmune spleen cells were suspended in Eagle's Minimal Essential Medium with Hanks' Balanced Salt Solution, supplemented with glutamine, nonessential amino acids, and sodium pyruvate with 10% FCS. 35 × 10-mm Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) were employed for culture, and each contained 2.0 ml of the suspension, $\cong 2 \times 10^7$ cells. The dishes were incubated for 20 h at 37°C in a humidified, 5% CO_2 atmosphere, to allow regrowth of surface Ig.

B and T lymphocytes from the cultured cells were separated after rosette formation with TNP-SRBC and the rosette-forming cells were later plaqued. Rosettes were made by a modification of the method of Parish et al. (4). Briefly, a 0.2-ml cell suspension ($\cong 2 \times 10^7$ cells) was mixed with 0.2 ml 10% (vol/vol) TNP-SRBC and centrifuged at 700 *g* for 5 min at 4°C. 4 ml PBS prewarmed to 20°C was added, and the pellet gently resuspended. The resulting suspension was then carefully layered onto 4.0 ml Ficoll-Hypaque, prewarmed to 20°C and centrifuged at 2,000 *g* for 20 min at 20°C. The separating medium was 10% Ficoll (Pharmacia Fine Chemicals, Piscataway, N. J.) wt/vol, and 9.6% Hypaque (Winthrop Laboratories, New York) vol/vol. After centrifugation, TNP-SRBC in the pellet were lysed with a buffered NH_4Cl solution at 4°C (5), and the remaining lymphocytes were washed extensively before plaque assay. These cells were 98% Ig-bearing by Fl-anti-Ig determination.

Statistical Analysis. PFC/10^6 viable cells were determined for each avidity group and geometric means and standard errors calculated. As each experimental group was composed of splenic aliquots from each hyperimmune mouse, the paired t test was used to test significance. Each experiment shown is one of two replicate studies.

Results

Modification of Hyperimmune Cell Sensitivity to Suppression. The inhibition of high but not low avidity PFC by carrier-activated T lymphocytes added at assay (1) suggests that high avidity membrane Ig, unlike that of low avidity PFC, might bind hapten carrier, and thus provide a recognizable determinant for the suppressing cell. If this speculation is accurate, hyperimmune cells might be sensitized by incubation with hapten carrier but protected by anti-carrier antibody.

In the first experiment, spleens were removed from mice primed 10 wk before with DNP-KLH in CFA and boosted 4 days before assay with DNP-KLH in saline. Splenic aliquots were incubated on ice for 1 h with 10 μg/ml of KLH, DNP-EA, or DNP-KLH. The cells were washed twice with antigen-free medium, and the hyperimmune cells were incubated for 90 min at 37°C in the presence of 10×10^6 KLH ATC just before plaque assay. Fig. 1 A presents groups which were preincubated with antigen but did not receive ATC, and demonstrates that antigen administration alone does not affect the avidity profile. Total PFC/10^6 are also unaffected. In contrast, the addition of ATC without antigen provokes a marked high avidity suppression (Fig. 1 B, $P < 0.001$ for avidity subgroup <8; $P < 0.05$ for 7–8) and a significant decrease in total PFC/10^6 cells. The preincubation of hyperimmune cells with 10 μg/ml KLH or DNP-EA before ATC treatment has no effect on total PFC/10^6 or the avidity profile. However, preincubation with DNP-KLH depresses the ATC-modulated response significantly ($P < 0.01$ for avidity subgroup 6–7; $P < 0.05$ for total PFC/10^6). In all experiments presented here, both direct and indirect PFC were measured. In no case, however, were the direct PFC responses of the experimental groups different from controls, so only indirect PFC are presented.

In the second experiment, DNP-KLH hyperimmune splenocytes were preincubated in a 1.0-ml volume for 1 h at 4°C with 0.13 mg anti-KLH or 0.17 mg normal Ig. After washing twice, these cells were incubated with 10^7 KLH ATC. Table I demonstrates that preincubation with antibody had no influence on the normal secondary response (groups 1–3), but with the addition of ATC, PFC in avidity subgroup <8 are significantly inhibited (group 4: $P < 0.001$) even after preincubation with guinea pig Ig (group 6: $P < 0.001$). On the other hand, preincubation with anti-KLH (group 5) completely protects from ATC suppression.

Suppression Blockade by Soluble Carrier. The previous experiments demonstrate as predicted that KLH does not influence suppression when preincubated with DNP-KLH hyperimmune cells. On the other hand, function of carrier-specific suppressor T cells might be altered by soluble KLH.

KLH-primed ATC were suspended in medium containing 10 μg/ml KLH, DNP-KLH, EA, or DNP-EA, and left on ice for 1 h. The cells were then washed twice and 8×10^6 ATC were incubated with DNP-KLH hyperimmune splenocytes; Table II gives the data obtained at PFC assay. High avidity PFC, given

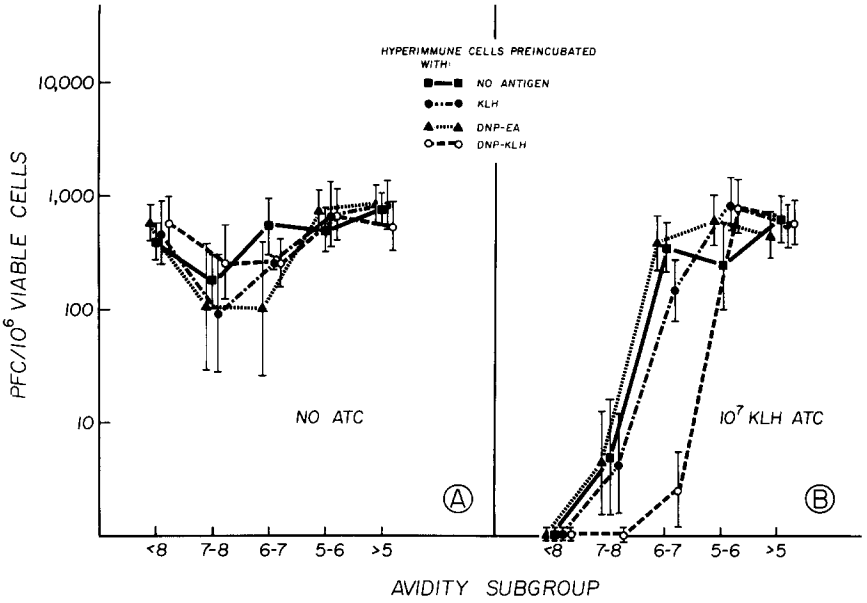

FIG. 1. Modification of suppression by preincubation of hyperimmune cells with antigen. Mice were given 100 μg DNP-KLH in CFA in the thighs, followed 10 wk later by 100 μg DNP-KLH in saline i.p. Just before PFC assay 4 days later, immune spleen subfractions (about 8×10^6 cells) were preincubated with no antigen, 10 μg/ml KLH, 10 μg/ml DNP-EA, or 10 μg/ml DNP-KLH. After washing, these cells were (B) or were not (A) incubated for 90 min at 37°C with 10^7 KLH ATC. Total indirect PFC/10^6 were significantly reduced for all groups receiving ATC ($P < 0.01$) and of these, preincubation of hyperimmune cells with DNP-KLH further reduced total PFC/10^6 ($P < 0.05$). Direct PFC were unaffected.

TABLE I
*Protection against Suppression by Anti-Carrier Antibody; Preincubation with Hyperimmune Cells**

Group	Immunoglobulin pulse	KLH ATC	Indirect PFC/10^6 viable cells‡		
			Total	<8	>8
1	–	0	4,476(1.12)	656(1.32)	3,673(1.16)
2	anti-KLH	0	5,290(1.09)	798(1.36)	4,207(1.15)
3	NGP	0	4,745(1.19)	879(1.17)	3,792(1.22)
4	–	10^7	3,332(1.15)**	0 ***	3,332(1.15)
5	anti-KLH	10^7	5,013(1.13)	1,267(1.21)	3,709(1.11)
6	NGP	10^7	3,886(1.20)	0 ***	3,886(1.20)

* Primary immunization, 100 μg DNP-KLH in CFA; secondary, 100 μg DNP-KLH in saline, 8 wk later; just before assay 4 days later, 10^7 hyperimmune splenocytes were incubated for 1 h, 0°C with 0.13 mg/ml anti-KLH Ig or 0.17 mg/ml normal guinea pig (NGP) Ig, then washed; KLH ATC were added, and incubated 90 min at 37°C.

‡ Geometric means, and in parentheses, standard error factors. Significance, denoted as *($P < 0.05$), **($P < 0.01$), or ***($P < 0.001$), was computed by paired t test, comparing vs. group 1.

for avidity subgroup <7, show complete protection from suppression when ATC were preincubated with KLH (group 3). Excellent high avidity suppression ($P < 0.05$) was observed for all other ATC preincubations (groups 2, 4–6).

KLH also provokes a dose-dependent blockade of suppression when added to

TABLE II

*Specificity of Inhibition of ATC Function by Antigen Preincubation with ATC**

Group	Antigen pulse	KLH ATC	Indirect PFC/10^6 viable cells‡		
			Total	<7	>7
1	0	0	3,867(1.33)	710(1.71)	3,064(1.29)
2	0	8×10^6	3,097(1.24)*	33(2.63)*	2,793(1.23)
3	KLH	8×10^6	3,899(1.25)	675(1.12)	3,208(1.28)
4	DNP-KLH	8×10^6	3,259(1.29)*	13(1.21)***	3,245(1.29)
5	EA	8×10^6	3,540(1.26)	31(2.43)*	3,442(1.25)
6	DNP-EA	8×10^6	3,833(1.26)	119(3.77)*	3,442(1.28)

* Primary immunization, 100 μg DNP-KLH in CFA; secondary immunization, 100 μg DNP-KLH in saline, 10 wk later; 4 days later, just before assay, KLH ATC were incubated for 1 h, 0°C with 10 μg/ml KLH, DNP-KLH, EA, or DNP-EA, then washed; 8×10^6 ATC were added to 10^7 hyperimmune splenocytes and incubated for 90 min at 37°C.

‡ Geometric means, and in parentheses, standard error factors. Significance, denoted as *($P < 0.05$), **($P < 0:01$), or ***($P < 0.001$), was computed by paired t test, comparing vs. group 1. Of groups 3–6, only group 3 was significantly different from group 2 ($P < 0.01$).

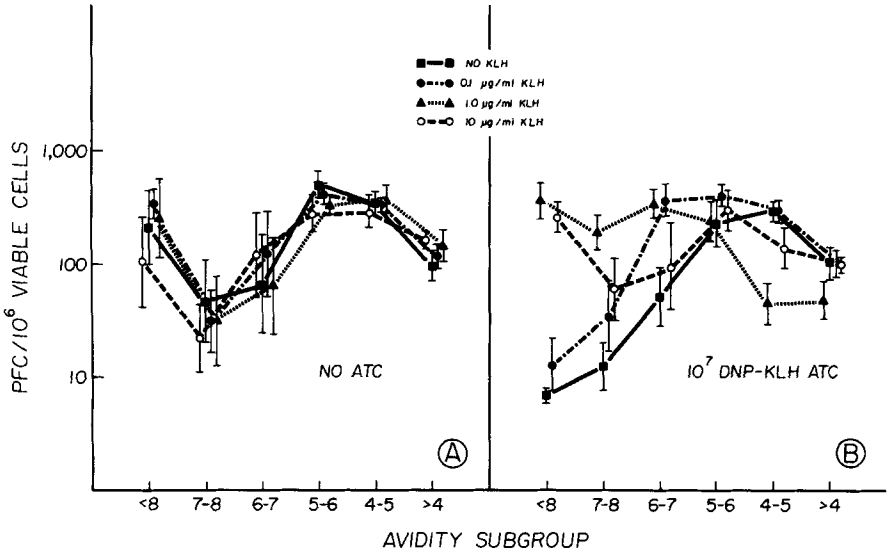

FIG. 2. Dose-dependent inhibition of suppression by soluble carrier. The primary immunization, 100 μg DNP-KLH in CFA, was given 7 wk before the secondary of 100 μg DNP-KLH in saline. Just before assay 4 days later, $\cong 9 \times 10^6$ hyperimmune spleen cells were mixed (B), or not mixed (A) with 10^7 DNP-KLH ATC, suspended in varying concentrations of KLH, and incubated for 90 min at 37°C. Total indirect PFC/10^6 were reduced only for the group incubated with ATC but without KLH ($P < 0.01$). Direct PFC were unaffected.

the ATC-hyperimmune cell incubation mixture. Hyperimmune splenocytes were mixed with 10^7 DNP-KLH ATC in a total volume of 1.0 ml containing 0, 0.1, 1, or 10 μg of KLH per ml, incubated at 37°C for 90 min, and then assayed. Fig. 2 shows that KLH did not influence the response when ATC were not present (A). ATC alone produced an exceptional deletion of high avidity PFC ($P < 0.001$ for avidity group <8; B). The administration of 0.1 μg/ml KLH

TABLE III

Loss and Recovery of Suppressibility after Pronase Treatment of Hyperimmune Cells

Immune splenocyte pretreatment*					Indirect PFC/10^6 viable cells‡		
Group	Pronase	Culture for Ig regrowth	DNP-KLH pulse	KLH ATC	Total	<8	>8
1	0	0	0	0	3,359(1.28)	482(1.31)	2,859(1.29)
2	0	0	0	10^7	2,767(1.33)*	10(1.23)***	2,755(1.33)
3	+	0	0	0	2,266(1.37)**	209(2.23)	1,852(1.42)*
4	+	0	0	10^7	2,156(1.37)**	226(2.37)	1,659(1.42)*
5	+	+	0	0	72,277(1.67)	15,662(1.96)	55,458(1.60)
6	+	+	0	10^7	52,949(1.37)	13,607(1.26)	36,547(1.56)
7	+	+	10 μg/ml	0	59,889(1.65)*	10,934(1.76)	41,512(1.74)
8	+	+	10 μg/ml	10^7	45,958(1.68)*	164(1.74)***	45,766(1.68)

* Primary immunization, 100 μg DNP-KLH in CFA; secondary, 100 μg DNP-KLH in saline, 6 wk later; immediately before assay 4 days later, hyperimmune spleen cells were treated with pronase, then incubated for 90 min at 37°C with 10^7 KLH ATC. One set of pronase-treated hyperimmune cells, before incubation with ATC, were placed in culture for 20 h at 37°C in 5% CO_2; the population was rosetted with TNP-SRBC, then incubated for 1 h on ice with 10 μg/ml DNP-KLH; after washing the hyperimmune cells twice, 10^7 KLH ATC/ml were added, and incubation proceeded for 90 min at 37°C. Approximately 8×10^6 hyperimmune cells comprised each group. Total PFC recovery from group 3 to group 5 ≅30%.

‡ Geometric means, and in parentheses, standard error factors. Significance denoted as *($P < 0.05$), **($P < 0.01$), or ***($P < 0.001$), was computed by paired t test, comparing groups 2–4 vs. 1 and 6–8 vs. 5.

with ATC did not diminish this suppression. Incubation with 1 or 10 μg/ml KLH, on the other hand, produced entirely normal avidity profiles.

Pronase Treatment of Hyperimmune Cells. The ability of DNP-KLH to preferentially sensitize high avidity PFC to KLH ATC implicates hapten-specific membrane Ig in the suppression. This idea, as well as the correlate that hapten carrier is required for suppression, was tested in the following manner.

Hyperimmune spleen cells removed from mice primed 6 wk and boosted 4 days earlier with DNP-KLH were optionally treated with pronase according to the procedure given in Materials and Methods, and then incubated with 10^7 KLH ATC (Table III). Other aliquots of pronase-treated cells were cultured for 20 h to allow membrane protein regrowth, treated with TNP-SRBC, and their rosettes were isolated. The cell population freed of erythrocytes was optionally suspended in 10 μg/ml DNP-KLH on ice for 1 h, washed twice, and then incubated with 10×10^6 KLH ATC. Pronase treatment alone (Table III, group 3) and antigen incubation of the cultured cells (group 7) produced minor, avidity nonspecific PFC depression. In our experience, this is not unusual for extended experimental manipulation. Pronase treatment before ATC administration (group 4) completely protects high avidity PFC from suppression by KLH suppressor cells (group 2; $P < 0.001$ for avidity subgroup <8). Moreover, pronase-treated hyperimmune cells do not recover suppressibility with culture (groups 5 and 6), as PFC for subgroup <8 demonstrates, unless the cells are incubated with DNP-KLH before mixing with KLH ATC (groups 7 and 8). This result strongly implies that the target for KLH-specific suppression in untreated splenocytes is cell-bound hapten-carrier conjugates.

Loss of Suppressor Carrier Specificity. It is shown elsewhere (1) that EA ATC do not suppress DNP-KLH hyperimmune cells. However, this carrier specificity may not depend completely on differences between EA- and KLH-activated T cells. Indeed, carrier specificity may simply mirror the identity of

TABLE IV
*Antigen-Mediated Shift in the Carrier Specificity of Suppression of DNP-KLH-Primed Splenocytes**

Group	Antigen pulse	EA ATC	Indirect PFC/10^6 viable cells‡		
			Total	<8	>8
1	0	0	3,108(1.43)	705(2.37)	2,191(1.28)
2	EA	0	2,931(1.28)	675(1.28)	2,208(1.21)
3	DNP-EA	0	3,360(1.15)	852(1.40)	2,324(1.21)
4	EA + DNP-KLH	0	2,940(1.21)	124(2.32)	2,532(1.25)
5	0	10^7	2,800(1.21)	819(1.14)	1,851(1.41)
6	EA	10^7	2,869(1.25)	1,189(1.25)	1,652(1.28)*
7	DNP-EA	10^7	2,178(1.18)**	13(1.21)***	2,165(1.18)
8	EA + DNP-KLH	10^7	3,003(1.17)	558(2.26)	1,949(1.75)

* Primary immunization, 100 μg DNP-KLH in CFA; secondary, 100 μg DNP-KLH in saline, 6 wk later; just before assay 4 days later, 8×10^6 immune spleen cells were preincubated with 10 μg/ml EA, DNP-EA, or 10 μg/ml each of EA and DNP-KLH for 1 h at 37°C. The cells were washed twice, then incubated with 10^7 EA ATC for 90 min at 37°C.

‡ Geometric means, and in parentheses, standard error factors. Significance, denoted as *($P < 0.05$), **($P < 0.01$), or ***($P < 0.001$) was computed by paired t test. Comparisons were made between groups preincubated with identical Ag preparations.

the hapten-carrier conjugate available to ATC and high avidity antibody-secreting cells. This possibility was evaluated as follows.

DNP-KLH hyperimmune splenocytes were preincubated in a total volume of 1.0 ml with 10 μg/ml EA, DNP-EA, or 10 μg/ml of both EA and DNP-KLH. After 1 h at 4°C, these cells were washed twice, incubated at 37°C for 90 min with or without 10^7 EA ATC, and assayed. Table IV shows that the inclusion of antigen alone (groups 2–4) does not significantly affect total or high avidity PFC. In the presence (group 6) or absence (group 5) of EA, EA ATC also do not influence the response. Similarly, the addition of DNP-KLH to EA in the preincubation mixture (group 8) has no effect. On the other hand, preincubation with DNP-EA (group 7) provokes significant reductions in both total PFC/10^6 ($P < 0.01$) and PFC/10^6 in the <8 avidity subgroup ($P < 0.001$).

Discussion

A suppressor mechanism consistent with these data requires hapten carrier on the surface of high avidity B cells, presumably bound by membrane Ig. Evidence to support this hypothesis is reasonably strong. The in vitro exposure of DNP-KLH hyperimmune cells to DNP-KLH but not KLH or DNP-EA, sensitizes the population to KLH ATC, producing an avidity-dependent increase in the observed suppression. In a related experiment, DNP-KLH hyperimmune cells become sensitive to EA ATC (contrary to the normal carrier specificity [1]) if hyperimmune cells are preincubated with DNP-EA but not EA alone, nor EA mixed with DNP-KLH. Thus, we propose that high avidity B cells recognize and bind specific haptenic determinants of the hapten-carrier conjugate, and thereupon gain surface carrier determinants which then serve as targets for carrier-specific suppressor T cells and/or their soluble products, resulting in depression of antibody secretion. As further support of this idea,

DNP-KLH hyperimmune cells are protected from KLH ATC by prior incubation with anti-KLH but not normal guinea pig Ig. Moreover, KLH ATC activity is specifically blocked by incubation with KLH (but not DNP-KLH) before mixture with target cells. This dichotomy favors a theory of physical blockade of suppressor molecules by KLH (presumably DNP-KLH bound to suppressor would be an active complex), although the carrier might also signal an alteration in suppressor cell function. KLH produces a dose-dependent emergence of high avidity PFC when added to the ATC-hyperimmune cell mixture, and lower avidity suppressible PFC are first protected. This finding is consistent with the concept that lower avidity PFC, possessing fewer targets for suppressor binding, would first escape a suppression threshold in a suppressor-limited system. Finally, and most impressive, is the loss of sensitivity of hyperimmune cells to ATC by prior treatment with pronase, suggesting the importance of membrane-bound proteins. In our hands, <5% of the cells bear Ig after this treatment, but recovery of membrane Ig occurs after 24 h in tissue culture (3), shown here by excellent concentration of PFC among immune rosetting cells after culture. Nevertheless, such DNP-KLH hyperimmune cells are not sensitive to KLH ATC, unless these cells are first incubated with DNP-KLH.

These results imply that hapten carrier normally remains at the surface of high avidity anti-DNP PFC for at least 4 days after boost. Presumably this requires both antigen and high affinity Ig. Although there has been difficulty in demonstrating membrane Ig on PFC (6, 7), McConnell (8), Nossal and Lewis (9), and Greeley and Scott (10) have largely resolved these discrepancies with the finding that membrane Ig decreases with time after the secondary immunization. Unlike McConnell, however, Bankert et al. (11) were unable to demonstrate rosetting indirect PFC. In agreement with McConnell (8), Nossal and Lewis (9) concluded that 90% of 4-day secondary PFC possess membrane Ig, compared to 40% of 23-day secondary PFC. High avidity antibody-secreting cells most likely have high avidity membrane Ig (12), consistent with the mechanistic theory presented here for T-cell–mediated suppression.

The question remains, however, if hapten carrier can be found at the PFC surface. Bystryn et al. (13) concluded that antigen may bind to cells in the secondary response even in the presence of high concentrations of circulating antibodies. In their experiments, DNP-bovine serum albumin still bound specifically to MOPC 315 cells when a 1,000-fold molar excess of soluble MOPC 315 Ig was included. Their expectation for the circulating antibody to circulating antigen ratio in the secondary response was <100. On the other hand, primed antigen-binding cells (not selected for antibody secretion) shed or endocytose antigen-membrane Ig complexes within 2 h of 37°C incubation in vitro (3). Still, small but functionally significant amounts of surface antigen may be protected from breakdown and elimination, as occurs in macrophages (14). Otherwise, lymphocyte surface-localized antigen would theoretically depend on the continual presence of antigen in the lymphocyte microenvironment. Antigen localizes in splenic follicles, found long after circulating antigen levels are quite low (15, 16). In fact, Ingraham (17) found 4% of a secondary immunization of ^{35}S-sulfanilic acid–azo-bovine γ-globulin in the spleen many weeks after boost. Moreover, lymphocytes appear at least partly responsible for this splenic

localization since thoracic duct drainage reduces follicular antigen uptake, a reduction only partly relieved by passive immunization (18). Nevertheless, a demonstration of antigen at the surface of hyperimmune cells has not been reported; in fact, Aldo-Bensen and Borel (19) were unable to show significant binding of anti-DNP antibody to spleen cells of mice given four weekly injections of DNP-KLH i.v. Conceivably, these results may reflect a very small population of high avidity cells (in view of the immunization procedure) concealed in a relatively high background response. Present attempts in this laboratory are focusing on the demonstration of KLH determinants at the surface of DNP-specific PFC.

Given the crucial role of antigens in mediating suppressor T-cell activity, other aspects of antigenic immunoregulation should be reconsidered. For example, antigens may well mediate carrier-specific helper T-cell and hapten-specific precursor communication. Particularly relevant is the recent work of Feldbush and van der Hoven (20), and Celada et al. (21), who showed that a high dose secondary immunization resulted in large amounts of low affinity antibodies. This result is reminiscent of increased T-cell suppression among hyperimmune splenocytes presensitized with hapten carrier. Unpublished data from our laboratory also suggest that the secondary immunization dose may influence the extent of high avidity suppression in the in vivo suppressor T-cell system we reported previously (22). On the other hand, since Feldbush and van der Hoven (20) determined serum antibody affinity, it is unclear whether the reciprocal influence of high antigenic challenge on affinity reflects deletion of high affinity antibodies, or a vast increment of lower affinity populations. In any event, antigenic modulation of the secondary response, acting via suppressor T cells, could provide effective immune regulation, perhaps limiting immune complex formation in the animal.

The possible relevance of this in vitro suppressor T-cell activity to tolerance is intriguing. The system is certainly unrelated to antibody-mediated tolerance (23), helper T-cell unresponsiveness (24, 25), and the immediate tolerance induced by polymeric antigens in spleen cells responding to DNP-Ficoll (26). Nevertheless, antigen-dependent T-cell suppression may interrelate the loss of high affinity antibodies in partially tolerant animals (27–30), the plausible role of suppressor cells in tolerance (31–33), and the firm demonstration of tolerogen at the surface of tolerant B cells (19, 34). Considering this information in detail, Andersson et al. (30), as well as Rajewsky and Brenig (35) found that high dose but not low dose tolerance results in a decline of antibody avidity. Sanfilippo and Scott (24, 25) have shown that helper-cell unresponsiveness does not provoke a shift in antibody avidity. Moreover, Bell and Shand (31) demonstrated a T-dependent avidity suppression by tolerant cells. In another experiment (36), these authors gave tolerant rats primed T or normal T and B cells, plus an antigen challenge. They found that a T-helper cell deletion was indeed part of tolerance, but that the high avidity B-cell response was irreversibly deleted, and could not be reconstituted with immune or normal T cells. Furthermore, primed B-memory cells are inhibited upon transfer to tolerant rats (31). Whereas other reports would tend to distinguish the suppressing from the tolerizing event (37), this conclusion rests on the presumption that

suppressed B cells would quickly be released if the inhibitor were removed; transferred T cells were only suppressive if transferred soon after the induction of tolerance. Finally, although Aldo-Benson and Borel (19, 34) have not investigated T-cell involvement or high avidity deletion in their tolerant B-cell population, these cells have been shown to carry surface-localized tolerogen (hapten carrier). Precisely like the suppressor T-cell system reported here, they find release from this tolerance by anti-carrier antibody (38).

In view of the preceding data, we propose that suppressor T cells are possibly involved in high zone tolerance, recognizing carrier determinants of antigen localized at the surface of high avidity B cells. On the other hand, we do not discount the involvement and importance of the tolerogen, whose chemical structure may facilitate continual lymphocyte surface presentation. Moreover, at least with some tolerogens (26), accessory cells are probably not required for tolerance induction.

The crucial direction for investigation is the intracellular biology of suppression. At the level of the suppressor T cell, preliminary evidence suggests that ATC sonicates are as effective as the intact cell. Such suppressor molecules specific for a single carrier may reflect a variety of idiotypic specificities, and demonstrates strong or weak histocompatibility requirements as found in the suppressor T-cell systems of Taniguchi et al. (39–41) and Kapp (J. Kapp, personal communication), respectively. The intracellular events resulting in functional deletion of high avidity PFC are also of considerable interest; membrane Ig may focus suppressor molecules at a proximate membrane receptor or permit concentration of the factor in the cellular microenvironment, allowing sufficient cellular uptake for suppression. Finally, the reversibility of suppression has important implications for our postulated role of suppressor cells in tolerance, and indeed, in the humoral immune response as a whole.

Summary

Carrier-specific suppressor T cells can suppress antibody secretion by high avidity IgG plaque-forming cells (PFC) within 90 min in vitro. This process can be blocked by the inclusion of soluble carrier in the cell mixture or by the exposure of target cells to anti-carrier antibodies or pronase. Moreover, suppression can be augmented by PFC exposure to the soluble hapten-carrier conjugate. Finally, carrier specificity may be overcome by preincubation of the target population with a hapten-heterologous carrier before addition of heterologous carrier ATC. Thus, it is likely that high avidity suppression depends upon immunogen bound to the surfaces of antibody-secreting cells which serves as a target for suppressor cells or molecules.

Note Added in Proof. It has come to our attention that recent shipments of AKR/Nat mice are outbred. This has affected the suppressor effect described in this paper. AKR/J (The Jackson Laboratory, Bar Harbor, Maine), but not AKR/Nat, now show late-acting suppression.

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