ABILITY OF ANTIGEN-SPECIFIC HELPER CELLS TO EFFECT A CLASS-RESTRICTED INCREASE IN TOTAL Ig-SECRETING CELLS IN SPLEENS AFTER IMMUNIZATION WITH THE ANTIGEN*

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A striking feature of the immune system is its ability to respond specifically to an enormous number of different antigens. However, it has been known for many years, that, in addition to the formation of specific antibody, immunization may lead to "nonspecific" activation of B lymphocytes, reflected by either large increases in cells synthesizing Ig (1–3) or increased levels of Ig in the circulation (4, 5), both of which lack specificity for the eliciting antigen. Analysis of this phenomenon has revealed that the activation of cells producing antibody (AFC)[1] and cells synthesizing Ig lacking specific activity (IgFC), is closely linked, although the response kinetics of each may differ considerably (6). The close association between activation of AFC and IgFC is particularly evident in the experiments of Cazenave et al. (7) and Eichmann et al. (8) which show that the product of each cell type may possess common hypervariable regions. Whether or not cells producing nonreactive Ig and those synthesizing antibody are derived from the same precursors is still not resolved. Findings suggesting that concomitant synthesis of both Ig and antibody occurs in localized areas of the same cells, led Miller et al. (9) to suggest that AFC are in fact derived from differentiated immunocytes already synthesizing Ig lacking any reactivity. However, these results are in contrast to those of Urbain-Vansanten which show synthesis of antibody and Ig in different plasma cells (10).

One of the fundamental questions raised by these experiments is whether nonspecific activation of B cells into Ig-secreting cells is a general feature of the immune response following antigenic stimulation and, if so, how do these cells differ in their requirements for activation from the specific AFC precursors. In the present study, which employs the reverse plaque assay (11) to detect either total splenic plaque-forming cells (PFC) or cells secreting Ig of one particular class or subclass, experiments were

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[1] *Abbreviations used in this paper:* AFC, antibody forming cell; AHGG, aggregated human gamma globulin; BA, *Brucella abortus*; CFA, complete Freund's Adjuvant; dHGG, deaggregated human gamma globulin; IgFC, immunoglobulin forming cell; KLH, keyhole limpet haemocyanin; Pc, phosphorylcholine; PFC, plaque-forming cell; RαMIg, rabbit anti-mouse Ig SSS-III, type III pneumococcal polysaccharide; SRBC, sheep erythrocytes; TNP-Ficoll, trinitrophenyl$_{58}$-aminoethylcarbamylmethyl Ficoll; VH-C, variable and constant portions of Ig-heavy chain.

performed to investigate (*a*) the conditions under which "nonspecific" activation occurs, (*b*) whether this response is random or restricted to certain sub-populations of B cells, and (*c*) the nature of the interacting cell types involved.

The results to be presented indicate that the increase in Ig-secreting cells observed in the spleens of both adult and neonatal mice occurs following injection of T-dependent but not T-independent antigens, and that the process is not random but results from a preferential activation of cells secreting specific classes of Ig (predominantly IgG), an observation which appears to correlate with that seen for the isotype in the antigen-specific response. Furthermore, tolerance experiments lead to the conclusion that IgFC, like the AFC, are dependent on the generation of antigen-specific helper cells. These data are compatible with the existence of populations of helper T cells with recognition structures for idiotypic or isotypic determinants on Ig molecules and which are capable of collaboration with B lymphocytes of any specificity providing they express that Ig determinant.

Materials and Methods

Animals. 6- to 8-wk-old BALB/c female mice used in most experiments were obtained from Cumberland View Farms, Clinton, Tenn. BALB/c athymic mice (fifth backcross) and their littermates were purchased from Sprague-Dawley, Madison, Wis. The BALB/c mice used in the tolerance studies were raised at the National Institutes of Health Animal Production Unit, Bethesda, Md.

Preparation of Cells. Cell suspensions were prepared in balanced salt solution plus 5% fetal calf serum (Microbiological Associates, Walkersville, Md.) by mincing spleens through stainless steel grids. Clumps were removed by passage through glass wool.

Antigens and Immunization. Trinitrophenyl$_{58}$-aminoethylcarbamylmethyl-Ficoll (TNP-Ficoll [Ficoll, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.]) and type III pneumococcal polysaccharide (SSS-III) were injected i.p. at doses of 10 and 0.5 μg, respectively. These antigens were generously provided by Doctors D. Mosier and P. Baker. Anti-sheep erythrocyte (SRBC) responses were elicited by an i.p. injection of 5×10^8 SRBC; Phosphorylcholine coupled to keyhole limpet haemocyanin (Pc-KLH), a kind gift from Dr. Kim Bottomly, was injected i.v. in doses of 50 μg. In some cases, mice were first primed with an i.p. injection of 100 μg KLH alone or in Freund's complete adjuvant (CFA) several weeks before immunization with Pc-KLH. Aggregated human gamma globulin (AHGG) and the deaggregated form (dHGG) were prepared according to Chiller and Weigle (12). To immunize, 400 μg AHGG was given i.v. In some experiments, specific unresponsiveness was induced with 5 mg dHGG injected i.p. on day 0 and tolerance tested by challenge with 400 μg AHGG injected on days 21 and 31, respectively.

Plaque-forming Cell Assay. PFC were detected using the Cunningham and Szenberg (13) modification of the haemolytic plaque assay (14). Specific PFC against SRBC, SSS-III, and TNP were measured using unmodified or antigen-coupled SRBC as indicator cells. With the exception of TNP-SRBC, which were prepared according to Rittenberg and Pratt (15), the coupling of antigens to sheep erythrocytes was achieved using $CrCl_3$. Specific anti-HGG PFC were detected using HGG-coated goat erythrocytes (16). To measure total Ig-secreting cells, a reverse PFC assay (11) was used which employs sheep anti-mouse Ig-coupled SRBC (via $CrCl_3$) as targets and a polyvalent rabbit anti-mouse Ig (RαMIg) as a developer. PFC-secreting Ig of a particular class were scored by employing class-specific RαMIg as developers. The preparation and specificity of these reagents which were kindly given by Dr. R. Asofsky have been described previously (17). In all assays guinea pig serum absorbed with the appropriate targets provided the source of complement.

Analysis indicates that numbers of PFC are logarithmically distributed; therefore geometric means ± SE will be reported.

Results

Is an Increase in Ig-Secreting Cells a General Feature after Immunization with Antigens? Nonspecific activation of Ig has been observed in several species after injection of many antigens (1–8). To examine whether this is an inevitable consequence of primary immunization, a variety of T-dependent and T-independent antigens were compared for their ability to induce B cell activation into Ig-secreting cells. Results are shown in Fig. 1. The T-independent antigens TNP-Ficoll and SSS-III resulted in little or no increase in total splenic Ig-secreting cells, whereas 20- to 40-fold increases

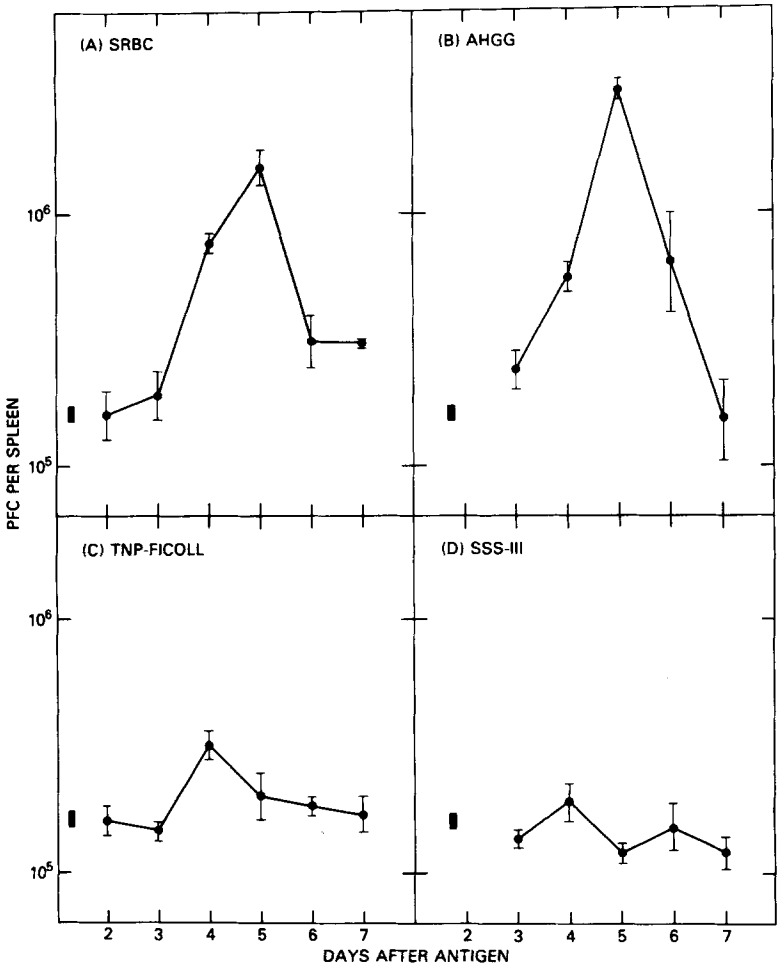

FIG. 1. Kinetics of the total Ig-secreting cell response in spleens following injection of 5×10^8 SRBC (*A*), 400 μg AHGG (*B*), 10 μg TNP-Ficoll (*C*), and 0.5 μg SSS-III (*D*). All antigens were given i.p. except for AHGG which was injected i.v. Points represent the geometric means ± SE of data pooled from three experiments (nine mice) in the case of Fig. 1 *A*, *C*, and *D* and two experiments in the case of Fig. 1 *B*. Vertical solid bars represent background levels of IgFC in spleens from a pool of 35 uninjected mice expressed as the geometric means ± 1 SE. For comparison, the peak total specific AFC responses in representative experiments were as follows: anti-SRBC, 186,000 ± 19,300 (day 4–5); anti-HGG, 22,600 ± 2,500 (day 6); anti-TNP, 46,700 ± 3,300 IgM (day 4), and anti-SIII, 31,500 ± 4,000 IgM (day 4).

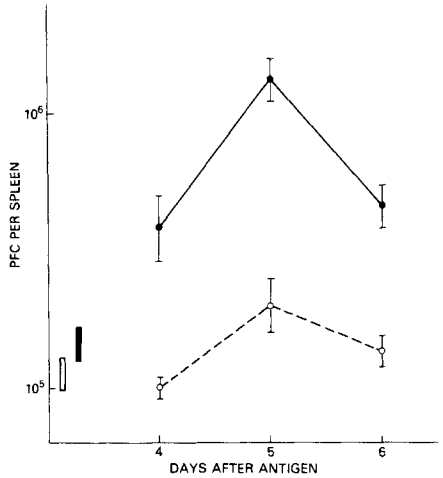

FIG. 2. Kinetics of the total splenic Ig-secreting cell response after immunization of BALB/c nude mice (○), and their littermate controls (●) with 5×10^8 SRBC. The data which are the results of a representative experiment are expressed as in Fig. 1. Open (nude) and closed (littermate) boxes represent the mean background levels obtained from six mice.

in total PFC were observed following injection of the T-dependent antigens SRBC and AHGG, respectively. The latter responses began to increase above background by day 4 after immunization, peaked at day 5, then rapidly decreased although background levels were not reached for several weeks. This peak response of Ig-secreting cells represented levels far in excess of the antigen-specific PFC responses. For example, total anti-SRBC PFC (including both direct and indirect) which peak at days 4–5, constituted a maximum of ~10% of the total Ig-secreting cells seen at day 5, whereas, in the case of AHGG, the peak level observed on this day was >1,000-fold greater than the HGG-specific PFC (peak days 6–7). It should be noted that very few indirect IgG HGG PFC occurred at day 5.

Because these observations do not eliminate the possibility that the T-dependent antigens, SRBC and AHGG, effected T-independent polyclonal activating properties, T-cell-deficient mice were immunized with SRBC to detect any direct effects of this antigen on B lymphocytes. The results presented in Fig. 2 showed that no significant increase above background occurred in athymic (nude) mice compared to the response of euthymic littermates. Similarly, no increases were observed in mice that had been thymectomized, irradiated, and reconstituted with bone marrow 2 mo before immunization (data not shown). These findings attest to the fact that the increase in Ig synthesis observed after immunization (Fig. 1) is T-cell dependent. The finding that background levels of splenic Ig-secreting cells in nudes is similar to that of their normal littermates is compatible with the findings that >90% of reverse PFC in normal spleens belong to the IgM class.

Analysis of the Ig Class Secreted by the Total Plaque-Forming Cell Population after Immunization. The results depicted in Fig. 1 provided a quantitative view of the phenomenon revealing Ig-secreting cells induced by T-dependent antigens. To provide more qualitative data, namely the number of PFC secreting Ig of particular classes or subclasses, the experiments were repeated using class-specific rabbit anti-mouse Ig

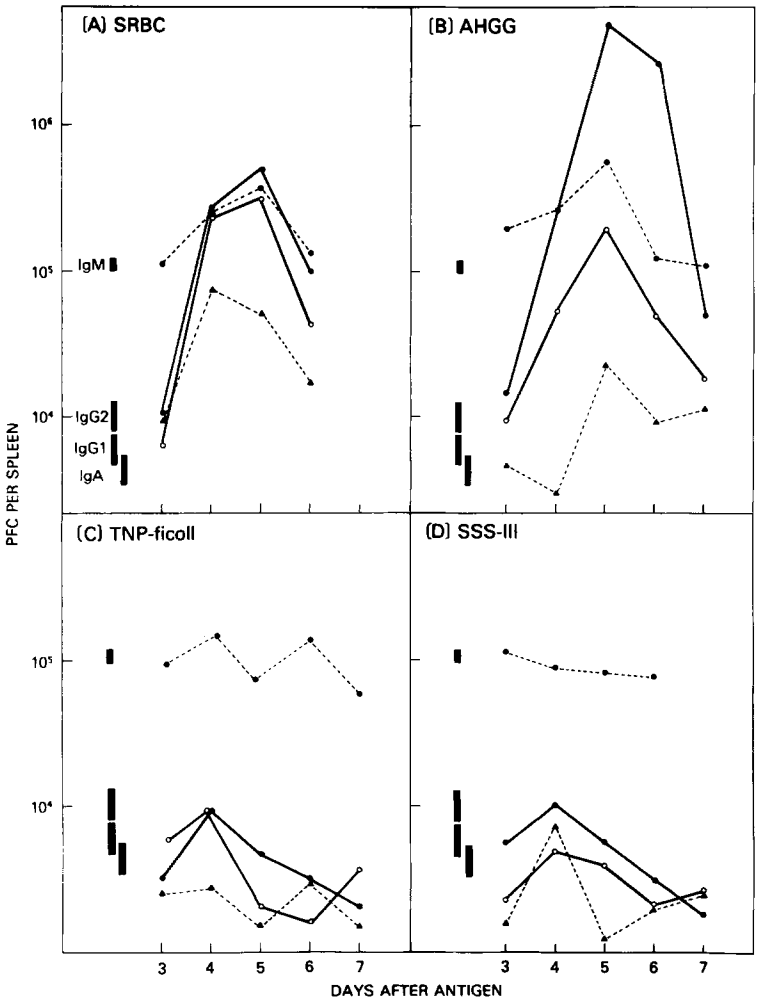

FIG. 3. Class-specific increases in total Ig-secreting cells per spleen of mice injected with SRBC (*A*), AHGG (*B*), TNP-Ficoll (*C*) and SSS-III (*D*) as a function of time. Points represent pooled values of two experiments in each case. Vertical solid bars indicate the total background IgFC in each class from pools of 20–35 normal mice; these values are expressed as geometric means. SE have been omitted for clarity; in no case did these exceed 0.236. (●---●) IgM; (●—●) IgG_1; (○—○) IgG_2; (▲---▲) IgA.

reagents as developers in the reverse plaque assay. Under such circumstances, it was observed that the large increases in total IgFC per spleen elicited by the T-dependent antigens SRBC and AHGG were due predominantly to a rise in the number of IgG_1, IgG_2 or IgA secretors, the exact ratio varying according to the antigen (Fig. 3 *A* and *B*). Thus, after immunization with SRBC, >40-fold increases in both IgG_1 and IgG_2 PFC were observed with 8- to 10-fold increases in the IgA class. In the case of AHGG, a primary response resulted in ~500- and 20-fold increases in IgG_1 and IgG_2, respectively, with no effect on IgA levels. All responses peaked at day 5. In addition, when specific and nonspecific PFC responses were compared after administration of SRBC and AHGG (Table I) a good correlation was found with respect to both the

TABLE I

Correlation between PFC Isotype and the Proportion of the Total PFC Contributed by Each Isotype in the Specific and Nonspecific Responses

Antigen*	Type of response	Class of PFC responses‡			
		IgM	IgG_1	IgG_2	IgA
SRBC	Specific	75,400	58,000	37,000	4,500
	(day 5)	(43%)	(33.2%)	(21.2%)	(2.6%)
	Nonspecific	351,000	595,000	532,000	110,000
	(day 5)	(22.1%)	(37.5%)	(33.5%)	(6.9%)
AHGG	Specific	75	21,604	519	205
	(day 6)	(0.03%)	(96.4%)	(2.3%)	(0.9%)
	Nonspecific	388,500	3,515,000	154,600	29,600
	(day 5)	(9.5%)	(86%)	(3.8%)	(0.7%)

* BALB/c mice were injected with 5×10^8 SRBC i.p. or 400 μg AHGG i.v.

‡ Average specific and nonspecific IgFC response from a pool of one to four experiments. The percentages shown indicate the portion of the total response detected represented by a given isotype.

heavy chain isotype produced and the percentage of the total response contributed by each isotype.

As expected, immunization with T-independent antigens resulted in no increase in the numbers of IgG_1, IgG_2 or IgA over background (Fig. 3 *C* and *D*). Despite this lack of effect on total PFC, specific AFC responses to TNP-Ficoll and SSS-III were always seen. It should be noted that a single injection of 0.3 ml of antilymphocyte serum prepared in horses had dramatic and selective effects on total IgFC identical to that seen with AHGG (data not shown), indicating that any heterologous serum treatment might have a marked T-cell-dependent influence on the B-cell compartment.

The Requirement for Antigen-specific Helper Cells in the Induction of Ig-Secreting Cells. Because the results of experiments described above demonstrated that T cells were required for the activation of both antigen-specific B cells and those of apparently unrelated specificities, it was important to determine whether this requirement involved helper cells specific for the antigen in question. To accomplish this, mice were first rendered specifically unresponsive to HGG by injecting the tolerogenic form of the antigen (dHGG) at a dose known to induce specific tolerance in both T and B lymphocytes (18). 3 wk later, one half of the tolerant mice and normal control animals were challenged with AHGG and increases in the total Ig-secreting cell levels were measured. As a specificity control, the remaining HGG-tolerant mice and equal numbers of normal animals were given SRBC. Assays for total PFC were done on day 5 after antigen administration.

The results presented in Table II show that mice treated with dHGG alone had unchanged levels of total background PFC at days 5 and 6 after injection and that challenging such animals with AHGG did not markedly increase the total IgFC responses compared to the response obtained in the nontolerant group given AHGG. In contrast, the levels of IgG_1- and IgG_2-secreting cells induced by SRBC were similar in both the HGG tolerant and nontolerant controls. Therefore the observed lack of an increase in IgFC in the tolerant mice was specific and suggests that in the absence of specific helper cells, nonspecific B-cell activation does not occur. A further point to be made from this experiment is that the phenomenon of induced Ig secretion is dependent on an immunogenic form of HGG (AHGG) and not simply the conse-

TABLE II

The Effect of Prior Induction of Tolerance on the Ability of AHGG to Stimulate Production of Ig-Secreting Cells‡*

Initial treatment of mice	Antigen	Numbers of class-specific IgFC			
		IgM	IgG_1	IgG_2	IgA
dHGG§	—	5.051 ± 0.98 (112,472)	4.079 ± 0.072 (12,000)	4.128 ± 0.276 (13,425)	3.976 ± 0.065 (9,461)
dHGG	AHGG	5.40 ± 0.06 (137,964)	4.894 ± 0.209 (78,337)	4.576 ± 0.13 (37,709)	4.105 ± 0.245 (22,667)
—	AHGG	5.427 ± 0.026 (267,238)	6.198 ± 0.105 (1,576,629)	4.938 ± 0.14 (86,667)	4.52 ± 0.05 (33,079)
dHGG	SRBC	5.496 ± 0.028 (313,575)	5.276 ± 0.133 (188,721)	5.215 ± 0.158 (164,034)	5.058 ± 0.07 (114,379)
—	SRBC	5.397 ± 0.028 (249,623)	5.011 ± 0.09 (102,479)	5.141 ± 0.019 (138,408)	4.82 ± 0.039 (66,113)
	Control	5.093 (124,000)	3.778 6,000	4.146 (14,000)	3.954 (9,000)

Data is presented as $\log_{10}$ Ig-secreting cells per spleen ± SE for each group; geometric means are in parentheses.

* Tolerance was tested by injections of 400 μg AHGG 21 and 31 d after treatment with dHGG. The mean anti-HGG response of seven mice was 2,550 PFC/spleen compared 56,595 PFC/spleen in nonpretreated mice.

‡ NIH BALB/c mice were rendered tolerant by an injection of 2.5 mg dHGG i.p. 3 wk later tolerant and normal control mice received 400 μg AHGG i.v. or 5×10^8 SRBC i.p. PFC assays on individual mice were done on day 5 after antigen.

§ PFC assays were done 5 d after dHGG.

quence of a trivial exposure to an heterologous globulin because dHGG did not effect a similar response.

Further evidence to support these conclusions was obtained from experiments in which mice were either immunized with AHGG 2–3 wk before SRBC or were given both antigens at the same time. The results indicate that (*a*) the simultaneous stimulation of two distinct helper cell populations after multiple immunizations leads to increased levels of class-specific Ig-secreting cells equal to the sum of the responses to each antigen alone (Table III), and (*b*) the levels of total PFC in mice immunized sequentially with AHGG and SRBC were not significantly different from control mice given SRBC alone (not shown), thus eliminating any large nonspecific effects of helper cells generated by prior exposure to an unrelated antigen.

The Effects of Carrier Priming on the Levels of Ig-Secreting Cells. Because the augmenting effects of adjuvants on helper T cell function is well known, it was of interest to examine the changes in total IgFC induced by CFA. Although a single injection of emulsified CFA alone resulted in only modest 5- to 10-fold increases in IgG secretion which lasted for 3 wk (not shown), the effects of adjuvant were much greater when it was used in conjunction with KLH in a carrier priming situation. Table IV compares the total class-specific PFC responses elicited by 50 μg of the T-dependent hapten-carrier conjugate Pc-KLH in unprimed mice, or in mice primed with either 0.2 ml of emulsified CFA, 100 μg KLH, or 100 μg KLH in CFA. Priming was done 6 wk before injection of the antigen. The results show that in unprimed mice, or in mice primed with CFA or KLH alone, five- to eightfold increases in IgG PFC were observed, which peaked on day 5. The elevated level of IgM PFC seen in the primed group on day 5

TABLE III

Additive Effect of Two Antigens Given Simultaneously on the Level of Total IgG- and IgA-Secreting Cells

Antigen* injected	Exp 1			Exp 2		
	IgG_1	IgG_2	IgA	IgG_1	IgG_2	IgA
	Number of class-specific IgFC ($\times 10^{-3}$)					
AHGG	768	444	4	720	590	5
	568	98	10	530	720	10
	824	272	7	560	320	5
	(5.852 ± 0.05)‡	(5.358 ± 0.193)	(3.816 ± 0.116)	(5.777 ± 0.041)	(5.711 ± 0.106)	(3.799 ± 0.1)
SRBC	290	424	172	176	302	5
	63	96	32	428	644	238
	143	190	69	80	384	44
	(5.139 ± 0.192)	(5.296 ± 0.186)	(4.860 ± 0.211)	(5.260 ± 0.21)	(5.624 ± 0.097)	(5.063 ± 0.218)
AHGG + SRBC	1,040	716	188	1,350	1,039	225
	791	716	168	1,200	1,070	240
	1,288	664	261	920	1,030	230
	(6.008 ± 0.061)	(5.844 ± 0.01)	(5.305 ± 0.057)	(6.058 ± 0.049)	(6.020 ± 0.005)	(2.365 ± 0.008)
Controls	14	2	2	5	8	1
	(4.146)	(3.301)	(3.301)	(3.699)	(3.903)	(30)

* Mice received either 400 μg AHGG i.v. or 5×10^8 SRBC i.p. or both antigens simultaneously. IgFC were quantified 4 d later.

‡ Numbers in brackets show geometric mean ± SE for each group.

TABLE IV

*The Effects of Different Priming Regimen on the Total Ig-Secreting Cell Response to the Hapten-Carrier Conjugate Pc-KLH**

Day of assay	Priming injections	Number of class-specific IgFC			
		IgM	IgG_1	IgG_2	IgA
D4	—	5.199 ± 0.062	4.165 ± 0.069	4.331 ± 0.100	3.360 ± 0.059
		(158,224)	(14,612)	(21,418)	(2,289)
	CFA	5.399 ± 0.11	4.382 ± 0.082	4.149 ± 0.056	3.933 ± 0.167
		(250,848)	(24,081)	(14,108)	(8,572)
	KLH in CFA	5.656 ± 0.081	5.783 ± 0.132	5.016 ± 0.204	3.761 ± 0.087
		(453,067)	(606,896)	(103,659)	(5,768)
	KLH‡	5.285 ± 0.073	4.317 ± 0.16	4.001 ± 0.078	3.913 ± 0.108
		(181,007)	(20,769)	(10,026)	(8,193)
D5	—	5.365 ± 0.014	4.640 ± 0.133	4.556 ± 0.224	4.055 ± 0.125
		(231,750)	(43,648)	(35,954)	(11,352)
	CFA	5.674 ± 0.132	4.759 ± 0.056	4.534 ± 0.068	4.434 ± 0.089
		(472,188)	(57,387)	(34,199)	(27,144)
	KLH in CFA	5.715 ± 0.068	5.876 ± 0.072	4.766 ± 0.005	4.198 ± 0.132
		(518,982)	(751,115)	(58,300)	(15,767)
	KLH	5.227 ± 0.027	4.566 ± 0.054	4.404 ± 0.006	3.519 ± 0.042
		(168,695)	(36,782)	(25,328)	(3,301)
	Control	5.079	4.0	3.954	3.477
		(120,000)	(10,000)	(9,000)	(3,000)

Data is presented as $\log_{10}$ IgFC ± SE of each group; geometric means are in parentheses.

* Mice were primed with either 0.2 ml CFA emulsified in saline, 100 μg KLH, or 100 μg KLH in CFA. 6 wk later primed mice and unprimed controls were injected with 50 μg Pc-KLH. 3 mice/group were assayed for total secreting cells at days 4 and 5.

‡ The data from the KLH-primed group is from a separate experiment.

was a result of an abnormally high response by a single mouse. However, mice primed with KLH in CFA exhibited a marked synergistic response equivalent to a 60-fold increase in IgG_1, a 10-fold increase in IgG_2, and a 4-fold increase in IgM responses all of which usually peaked on day 4–5.

Similar effects using Pc-KLH as antigen have also been observed in preliminary in vitro experiments.[2] In this case, KLH plus CFA-primed T cells were mixed with normal spleen cells and stimulated with Pc-KLH or Pc-*Brucella abortus* (Pc-BA), a T-independent antigen. Responses were elicited only by the T-dependent carrier (KLH) and at day 6 IgFC belonged predominantly to the IgG_1 class (16,000 IgFC/culture) and to a lesser extent IgG_2 (1,300 IgFC/culture) demonstrating a class restriction similar to that observed in vivo. IgG levels in the Pc-BA and control cultures did not exceed 250 PFC/culture in these experiments.

Discussion

The aim of the present study was to examine parameters and possible mechanisms by which immunization with a variety of antigens results in an increase in total numbers of splenic IgFC which is far too great to be accounted by the number of antigen-specific PFC alone. The results presented extend the earlier findings of others (1–6) and demonstrate that the nonspecific increase in Ig-secreting cells is not a general feature of the immune response after immunization with any antigen as previously suggested (19) but occurs only with antigens capable of stimulating specific T cell help. Thus, T-dependent antigens as diverse as SRBC and soluble proteins induced nonspecific B-cell activation to varying degrees, while T-independent antigens such as TNP-Ficoll or pneumococcal polysaccharide induced specific antibody-forming cells but no significant numbers of IgFC. These data are in part confirmatory of the results previously described by Antoine et al. (20) although it should be noted that in these and other studies describing the phenomenon of nonspecific Ig stimulation antigens have been administered in CFA, an adjuvant which can itself elevate the numbers of IgFC and which quantitatively enhances the effect elicited by specific T-dependent antigens.

A second approach to show both specificity and T-cell requirements for the formation of IgFC was to utilize adult animals tolerized with dHGG. It was shown that such animals failed to demonstrate either AFC or increases in IgFC in response to a challenge with AHGG but responded normally, i.e., both AFC and IgFC, when challenged with SRBC. Recently Carelli et al. (21) reached similar conclusions in animals tolerized to either bovine serum albumin or HGG. An additional point which falls out of the studies using dHGG is that gamma globulins as antigens do not give rise to IgFC simply because of their antibody nature inasmuch as the tolerogenic form of HGG by itself caused no increase in the total Ig-secreting cell level (Table II). Thus, it appears that immunogenicity is a necessary criterion for the effect provided by T-dependent antigens and in this regard HGG is a favorable test system because the aggregated form of it can be highly immunogenic without the need of extraneous adjuvants.

Further support for the role of specific T cells in eliciting IgFC came from findings

[2] Y. Rosenberg and K. Bottomly, unpublished observations.

that simultaneous immunization with two antigens, and by inference the generation of two helper cell populations, resulted in additive effects in all classes of PFC. Levels of serum IgG, higher than those observed by a single antigen, were also seen by de Vos Cloetens et al. (19) after dual immunization of rabbits. With respect to the role of such helper cells in nonspecific B-cell activation, experiments were done to test the effects of preimmunization with AHGG 2–3 wk before injection with SRBC. These demonstrated that the HGG-specific T cells, once generated, could act directly or indirectly on only a proportion of B cells rather than nonspecifically influencing the B-cell population induced by SRBC.

A significant finding in the present study was that the stimulation of nonspecific B lymphocytes after antigen injection was not random but occurred in a class-restricted manner. The precursor populations preferentially stimulated nonspecifically were those which eventually secreted IgG and, in the case of SRBC, IgA as well. In most instances, the largest increases occurred in the IgG_1 subclasses of IgFC (Fig. 3) especially after immunization with AHGG in which case levels of >500-fold above background were seen at days 5 and 6. The increases in IgFC PFC were however much smaller (sevenfold less) after rechallenge with AHGG than that observed following the primary response given 2 wk earlier.[3] It is not clear why nonspecific IgM PFC levels were not greatly increased, considering the substantial direct anti-SRBC response observed and the ease with which IgM precursors can be stimulated to secretion by mitogens (22). One possibility is that direct anti-SRBC responses may require levels of help too low to stimulate nonspecific PFC, and in this way, may be related to the inability of T-independent antigens to induce the phenomenon of Ig secretion.

Perhaps the most intriguing aspect of these data is the apparent correlation between the isotypes, other than IgM, of the specific and nonspecific PFC responses. For example, the patterns of the SRBC ($IgG_1 = IgG_2 > IgA$)- and AHGG ($IgG_1 \gg IgG_2$)-specific and nonspecific increases were similar. In keeping with this correlation, the occasional mouse which failed to give indirect specific anti-SRBC also failed to show an increase in its total IgG PFC level.

Several mechanisms may account for the ability of antigen-specific helper cells to effectively induce, or collaborate with large numbers of B lymphocytes apparently lacking specificity for the antigen. First, it is possible that all Ig-secreting cells may indeed be specific for the antigen but are of too low affinity to be detected by antigen-specific assays (19, 23). This would however, assume a precursor frequency too large to be accommodated by estimations of the relative frequencies of B cells specific for any one antigen (24). In addition, experiments by Asofsky et al. (5) demonstrated that the nonspecific Ig induced by ferritin could not be removed on ferritin-coated columns. This procedure would have been expected to remove at least a proportion of the low affinity antibody specific for the antigen and thereby reduce the level of nonspecific Ig. Second, it could be postulated that lymphokines produced by a specific helper population after interaction with antigen are able to induce B cells nonspecifically. In this case, the data could only be explained by the assumption that B-cell-stimulating factors are class-specific, a situation for which no evidence exists at the present time. In this regard, recent studies in this laboratory have shown that during

[3] Y. Rosenberg and J. Chiller, manuscript in preparation.

infections of mice with malaria, a marked T-cell-dependent polyclonal B-cell activation occurs (25); however in this case where B-lymphocyte activation is probably mediated by lymphokines, all B cells are equally triggered regardless of their Ig class, so that no isotype preference is observed.[4]

Based on the present data, the apparent nonspecific T-B cooperation can best be explained by invoking a second Ig-specific helper mechanism similar to that proposed by Janeway et al. (26). The existence of helper cells which have the ability to recognize Ig determinants has been demonstrated in several systems. For example, T cells specific for idiotypic (27, 28), allotypic (29), or isotypic (30) determinants expressed on the Ig molecules of B cells have been described. In some of these experiments induction of memory B cells was shown to require signals from both carrier- and idiotype-specific helper cells (27). In the present study, the findings that would be compatible with the generation of a second helper cell are (*a*) the very large class-restricted generation of nonspecific Ig-secreting cells and (*b*) the marked synergy observed in the response to Pc-KLH in mice primed with both KLH and CFA. Presently it is not known what determinants are recognized by the proposed second helper cell, if there is a need for the two T-cell populations to be antigen-specific (31, 32, 33) or whether only the Ig-specific T cells are required to induce B cells nonspecifically.

Two models of nonspecific T-B collaboration which would explain the data invoke the generation of T helper cells capable of recognizing either isotypic or idiotypic determinants. In the first case, specific B cells expressing and eventually secreting Ig molecules bearing isotypic (or allotypic) determinants could stimulate Ig-specific helper cells, which in turn are able to recognize and trigger cells bearing that determinant regardless of their specificity. Accordingly, the class correlation between the specific and nonspecific responses would be a natural consequence of recognition of isotype by the second helper cell. However, the additive effects of two antigens are more difficult to explain using this model unless it is assumed that the interaction of antigen with the Ig receptor occurs in such a way that isotypic (or allotypic) determinants are presented in an antigen-specific manner.

A second model supposes that antigen injection may result in the generation of either idiotype-bearing helper cells which could collaborate with any idiotypically similar B cells (both specific and nonspecific) or alternatively, idiotype-specific helper cells which could trigger any B cell expressing the idiotype by virtue of complimentary receptors. Both types of idiotype interaction, which have been described experimentally by Eichmann et al. (34) in the anticarbohydrate response, can also explain the presence of idiotype on the Ig molecules synthesized by specific antibody-forming cells as well as those lacking reactivity for the inducing antigen (7, 8). Additive effects of two antigens would also be predicted from such interactions. However, the data showing class restriction is more difficult to interpret unless nonrandom joining of variable and constant regions of the Ig-heavy chain (V_H-C) occurs: similar to the restriction of V_H regions to particular IgG subclasses suggested by the responses to several carbohydrate antigens (35, 36).

At the present time, experiments are in progress to further characterize the mechanisms by which T-cell interactions occur after immunization which can result

[4] Y. Rosenberg, manuscript in preparation.

in 3% of splenic B cells producing IgG_1 PFC of unknown specificity and to examine whether the phenomena of class-restriction is under genetic control.

Summary

Immunization with antigens stimulates not only B lymphocytes secreting specific antibody but, in addition, results in the generation of very large numbers of splenic Ig-secreting cells which lack specificity for that antigen. The present report examined the nature of the antigens capable of eliciting this effect and the mechanisms whereby B cells could be nonspecifically activated. It is shown that the ability of T-dependent, but not T-independent antigens, to induce such increases requires the participation of T helper cells specific for the antigen so that any one antigen results in the activation of only a proportion of total B cells. Analysis of this nonspecific plaque-forming-cell response reveals that B cell activation is not random but occurs in a class-restricted manner. The magnitude of the increase and the isotype produced are shown to be characteristic of the immunizing antigen.

Based on the data presented, the apparent nonspecific T-B collaboration can best be explained by invoking a second Ig-specific helper mechanism in which helper cells capable of recognizing determinants on Ig molecules, e.g. isotype or idiotype, cause the stimulation of B cells of any specificity providing they express that determinant.

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