The regulation of the IgE immune response has been shown to be dependent upon the dose of the antigen employed for the induction of the primary response (1) as well as during secondary antigenic stimulation (2–5), persistent IgE responses being favored by low doses of antigen. Takatsu and Ishizaka, utilizing passive transfer experiments in mice, demonstrated that high doses of antigen prevented the development of antigen-specific IgE bone marrow-derived lymphocytes (B cells) in the spleens of the treated mice (3), caused a decreased carrier-specific helper activity of the thymus-derived lymphocyte (T cells) in the spleens (4), and that these effects were due to the induction of T suppressor cells by the high doses of antigen (5).

Maia et al. (2), on the other hand, postulated that IgE B cells can be made tolerant by the administration of high doses of antigen, and concluded that the differential susceptibility to antigen-induced suppression of IgE and IgG antibody responses was due to the fact that IgE B cells were more easily made tolerant than IgG B cells. Results of experiments reported by Lee and Sehon (6–8) supported that concept.

It has been well documented that the IgE antibody response is under a strong T-lymphocyte influence. Induction of an IgE response in mice requires the participation of T cells (9). Katz et al. (10) demonstrated that the IgE antibody response by B cells is more dependent on specific helper signals from T lymphocytes than IgG B cells. The ongoing IgE response is more sensitive to suppressor T-cell signals as a result of an increased susceptibility of IgE B cells to T-cell signals, as proposed by Katz et al. (10), or an increased susceptibility of the IgE T helper lymphocytes to T suppressor-cell signals, as proposed by Takatsu and Ishizaka (5).
A great deal of information has been obtained utilizing in vitro systems for studying the regulation of antibody production. The studies presented in this paper were designed to examine the regulation of the IgE immune response in vitro. In particular, we wished to study the role of antigen in the regulation of IgE antibody formation, to define the cellular site of such regulation, and to compare the IgE antibody response in vitro to the response in other immunoglobulin classes of antibody.

Materials and Methods

Animals. Female CBA/J, AKR/J, C3H/HeJ, and HRS/J (hairless) mice (6-10 wk old) were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Antigens. Hen egg albumin (EA), crystallized five times, and bovine gamma globulin (BGG), Fr II, were purchased from Miles Laboratories, Inc., Elkhart, Ind.

The sodium salt of 2,4-dinitrobenzene sulfonic acid (Eastman Kodak Co., Rochester, N. Y.) was used to couple dinitrophenyl (DNP) haptenic groups to EA and BGG (11). After extensive dialysis against distilled water, the molar ratios of DNP to protein of the conjugates were determined spectrophotometrically and found to be 3.2 DNP:1 EA and 56 DNP:1 BGG.

Mitogens. Lipopolysaccharide W (LPS) from Escherichia coli O127:B8, obtained from Difco Laboratories, Detroit, Mich., was dissolved in saline and placed in a boiling water bath for 30 min before use. Purified concanavalin A (Con A) was the gift of Dr. D. Behnke's laboratory (Department of Biochemistry, University of Cincinnati, Cincinnati, Ohio).

Immunization. All mice were immunized by intraperitoneal injection (i.p.) with EA or DNP-EA in aluminum hydroxide gel which had been prepared by the method of Levine and Vaz (1). Antigen doses were as described in the figure legends.

Cell Culture Systems. Spleens from mice were removed aseptically and made into single cell suspensions by pressing through 80-mesh stainless steel screens into Eagle's Minimal Essential Medium (MEM; Grand Island Biological Co., Grand Island, N. Y.), prepared as described by Mishell and Dutton (12), and supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). The resulting cell suspensions were washed once and resuspended in the MEM supplemented with 10% fetal calf serum (FCS; Reheis Chemical Co., New York). The cells were adjusted to 1.8-2.2 x 10^7 viable cells/ml, as determined by trypan dye exclusion, and 1-ml aliquots were placed in 35-mm plastic tissue culture dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) for culturing under Mishell-Dutton tissue culture conditions, as described previously (13).

Cellular proliferation in vitro was measured by assessing the incorporation of tritiated [3H]thymidine (New England Nuclear, Boston, Mass.) by spleen cells stimulated with LPS (10 μg) or Con A (2 μg). These concentrations of mitogens were found to be optimal under the experimental conditions employed. Spleen cells, or subpopulations of spleen cells, were adjusted to 4 x 10^5 viable cells/ml RPMI 1640 medium (Grand Island Biological Co.), supplemented with 5% heat-inactivated (56°C, 30 min) FCS. Cells were cultured in triplicate in 0.5-ml aliquots in plastic tissue culture tubes (model 2058; Falcon Plastics) in a humidified CO2 incubator (37°C). At the end of 24 h, each culture received 1 μCi [3H]thymidine, and culturing was continued for another 16 h. Cells were harvested for counting 3H, as described previously (13).

Cell Separation Techniques. Spleen cells were enriched for B cells by incubating them for 30 min at room temperature in a 1:10 dilution of anti-μ serum (prepared by injecting AKR/J mice weekly for 10 wk, i.p., with 10^9 thymocytes taken from C3H/HeJ mice), followed by incubation at 37°C in the presence of sterile guinea pig complement (C; Microbiological Associates, Bethesda, Md.). The optimal ratio of anti-μ serum to spleen cells for killing T cells had been determined previously by determining the optimal ratio for killing thymocytes.

The nylon wool technique, originally described by Julius et al. (14), as modified by Schwartz et al. (15), was used to obtain a splenic lymphocyte preparation depleted of adherent cells and B cells and thus enriched for T cells.

Spleen cells were separated into an adherent (A) cell population (presumed to be mainly macrophages) and a nonadherent (NA) cell population by allowing A cells to attach to plastic tissue culture dishes as described by Mosier (16). 1-ml aliquots of spleen cell suspensions (1.8-2.2
were placed in the tissue culture dishes and incubated at 37°C for 30 min in a plastic box, charged with a three-gas mixture (13), placed on a rocking platform (10 oscillations per min). The NA cell population was that removable by gentle washing of the bottom of the Petri dish. The A cell population was that which remained attached after two 1-ml washes with MEM, and was quantitated by counting 10 microscope fields of known area and extrapolating to the number of A cells/dish. The percentage of macrophages in the A and NA cell populations was determined by assessing phagocytosis of neutral red (17).

**Antibody Assay Methods.** Reaginic (IgE) antibody titers were determined by injecting 0.05-ml aliquots of twofold dilutions of cell cultures or supernates of cell cultures into the skin of HRS/J mice. Anti-EA and anti-DNP IgE titers were measured by challenging recipient mice 24 h later with EA (1 mg) or DNP-BGG (100 μg), respectively, in Evans blue dye (18). It was found that the antibodies detected by this technique were heat-labile (56°C, 4 h), long-term sensitizing, and thus, classifiable as mouse IgE (19). IgE titers were found not to vary by more than twofold, and unless indicated by standard error, a greater than twofold difference in titers is considered to be significant.

Anti-DNP IgM and IgG production in vitro was determined by the plaque-forming cell (PFC) assay, originally described by Jerne and Nordin (20), as modified by Rittenberg and Pratt (21) with trinitrophenol (TNP)-coated sheep erythrocytes. The cross-reactivity of anti-DNP antibodies with TNP has been demonstrated previously by others (22). Rabbit antimouse IgG (Miles Laboratories Inc.) diluted 1:100 in medium 199 (m199, Grand Island Biological Co.), was used to facilitate anti-DNP indirect (IgG) PFCs.

**Results**

**Anti-DNP IgE Production In Vitro.** To differentiate more clearly cell types involved in antibody regulation, we employed a hapten-carrier system, DNP-EA antigen, in our priming regimen.

Two priming doses (1 or 100 μg DNP-EA in 1 mg Al(OH)₃ gel) were examined to determine which schedule would allow the greater production of anti-DNP IgE in vitro. 11 days after primary immunization, the time of peak anti-DNP IgE production in CBA/J mice, was chosen as the point at which spleen cells would receive secondary antigenic challenge in vitro. The kinetics of anti-DNP IgE production in vitro by spleen cells cultured in the absence of antigen are shown in Fig. 1. Spleen cells from the mice primed in vivo with 1 μg DNP-EA produced higher titers of anti-DNP IgE (1:32) than did spleen cells from mice primed with 100 μg DNP-EA (1:4 peak titer). Peak antibody titers were observed after 4-5 days of culturing.

The production of anti-DNP IgE antibody by primed spleen cells cultured in the presence of DNP-EA is shown in Fig. 2. The amount of antibody detected decreased sharply as the concentration of DNP-EA in the culture was increased from 1 × 10⁻⁴ to 1 × 10⁻¹ μg/ml. The kinetics of IgE production by cultures stimulated with exogenous antigen were not altered, i.e., peak titers were detectable from day 3 to day 5 regardless of DNP-EA concentration.

It was found that the titers of anti-DNP IgE antibody in the supernates of 4- and 5-day tissue cultures were identical to the IgE titers of the whole cultures (including lymphocytes). In addition, the IgE antibody detected by injecting supernates for passive cutaneous anaphylaxis (PCA) determinations was equally as heat-labile as the IgE detected by injecting whole spleen cell cultures. Therefore, in all subsequent experiments, whole cultures were used to assay for anti-DNP IgE.

Test for Neutralization of Anti-DNP IgE by Antigen In Vitro. The next
FIG. 1. Kinetics of antihapten in vitro IgE production by spleen cells from CBA/J mice immunized with 1 µg (○—○) or 100 µg (●—●) DNP-EA in Al(OH)₃ gel. After 11 days, the spleen cells were placed in tissue culture in the absence of exogenous antigen. Triplicate cultures were harvested after 4 and 5 days in vitro and assayed in duplicate HRS/J mice for the presence of anti-DNP IgE by 24-h passive cutaneous anaphylaxis (PCA). Challenge antigen for the PCA test was DNP-BGG.

FIG. 2. Effect of adding antigen on production of anti-DNP IgE in tissue culture. Spleen cells from mice immunized 11 days earlier with 1 µg DNP-EA in Al(OH)₃ gel were placed in tissue culture on day 0 and were cultured without antigen (○—○) or in the presence of $1 \times 10^{-4}$ (△—△), $1 \times 10^{-3}$ (□—□), $1 \times 10^{-2}$ (○—○), or $1 \times 10^{-1}$ (■—■) µg DNP-EA. IgE titers shown represent the $\log_{10}$ heat-labile anti-DNP antibody titers of duplicate cultures assayed by 24-h PCA in duplicate HRS/J mice at the times indicated.
experiment was designed to determine whether the concentrations of antigen to be employed in in vitro experiments neutralized anti-DNP IgE. Hapten coupled to homologous carrier (DNP-EA), hapten coupled to heterologous carrier (DNP-BGG), or carrier alone (EA) were added on day 0 at appropriate concentrations to cultures containing mouse serum anti-DNP IgE antibody (diluted to 1:16–1:32, a titer approximating the maximum anti-DNP IgE titer attainable in tissue culture) alone or in the presence of 2 × 10⁷ normal (nonimmune) CBA/J splenic lymphocytes. The residual anti-DNP IgE titers, after 5 days of incubation under standard tissue culture conditions, are shown in Table I. None of the antigen (hapten and/or carrier) concentrations tested resulted in neutralization of the anti-DNP IgE antibodies. In addition, the presence of splenic lymphocytes did not alter survival or neutralization patterns of the IgE. Tissue-culture-produced IgE (in the EA in vitro system, data not presented) also showed a lack of neutralization by EA until the antigen concentration was ≥5 × 10⁻¹ µg/ml.

Test for Inhibition of Anti-DNP IgE Production by Hapten and/or Carrier In Vitro. The results of the preceding experiment indicated that any decrease in IgE production in vitro induced by antigen concentrations up to and including 1 × 10⁻¹ µg/ml could not be attributed to neutralization. In the next experiment, the ability of hapten or carrier alone to induce inhibition of antihapten IgE production was examined.

As shown in Fig. 3, maximum IgE production occurred in the absence of exogenous antigens. Hapten, whether coupled to homologous carrier (DNP-EA) or heterologous carrier (DNP-BGG) suppressed anti-DNP IgE production,
Fig. 3. Inhibition of in vitro IgE production by antigen. Spleen cells from mice immunized 11 days earlier with 1 µg DNP-EA plus Al(OH)₃ gel were cultured for 5 days in the presence of DNP-EA (●—●), DNP-BGG (△—△), or EA (■—■). Anti-DNP IgE titers shown represent the x log₂, based on duplicate cultures assayed in duplicate.

TABLE II
Separation of Spleen Cells into B-Cell-Enriched or T-Cell-Enriched Populations

| Experiment | Anti-θ + C' sensitivity of whole spleen (% lymphocytes killed) | Nylon wool effluent | Spleen cells non-adherent | Nonadherent cells killed by anti-θ + C' |
|------------|-------------------------------------------------------------|---------------------|--------------------------|----------------------------------------|
|            |                                                             |                     | %                        | %                                      |
| 1          | 32                                                          | 13                  | 85                       |
| 2          | 24                                                          | 8                   | 90                       |
| 3          | 29                                                          | 12.5                | 95                       |

whereas carrier (EA) alone had no enhancing or suppressing effect on anti-DNP IgE production in vitro.

Role of T Lymphocytes in Antihapten IgE Production or Suppression In Vitro. The contribution made by T cells to the production of hapten-induced inhibition of IgE production in vitro was next investigated.

Spleen cells from primed mice were depleted of T cells by anti-θ plus C' treatment (B cell enriched) or enriched for T cells by nylon wool passage. As shown in Table II, anti-θ + C'-sensitive spleen cells accounted for 29-34% of the CBA/J spleen cell population. The yield of cells from nylon wool columns ranged from 8 to 13% of the whole spleen, and the anti-θ + C' sensitivity of this T cell-enriched population was found to be 85-95%.

In control experiments, whole or enriched spleen cell populations were tested for their proliferative response (³H)thymidine incorporation) in vitro to mitogenic stimulation by 10 µg LPS, a B-cell mitogen, and to 2 µg Con A, a T-cell mitogen. The mean counts per minute (cpm) ± standard error (³H)thymidine incorporated were as follows (background cpm subtracted). Whole spleen cell cultures were stimulated by both the LPS (228,765 ± 914 cpm) and the Con A (272,459 ± 8,236 cpm). The B cell-enriched population responded well to LPS (243,805 ± 1,680 cpm) but poorly to Con A (~8,191 ± 1,730 cpm), whereas the T
cell-enriched population responded minimally to LPS (22,557 ± 351 cpm) but gave a good proliferative response to Con A (274,956 ± 16,670 cpm).

The anti-DNP IgE antibody production by primed whole spleen cell populations, B cell-enriched (anti-θ + C'-treated spleen), or B cell-enriched primed spleen cells reconstituted with T cell-enriched primed spleen cells (nylon wool effluent) was determined after 5 days of culturing in vitro. Results of the IgE determinations from two separate experiments were combined and are shown in Fig. 4. The magnitude of the anti-DNP IgE response by "B" cells or "B + T" cells cultured in the absence of exogenous antigen was not significantly different (x log₂ titers were 3.5) from that by the whole spleen cell population (x log₂ titer was 4.0). In addition, the pattern of hapten-induced inhibition of anti-DNP IgE production was essentially the same for all three cell populations.

Role of A Cells in the Production and Inhibition of Anti-DNP IgE In Vitro. A cells have been shown to play an important role in immune responses by murine splenic lymphocytes in vitro (23-25). To determine the role of A cells in
the production or inhibition of anti-DNP IgE antibody in vitro, primed spleen cells were depleted of A cells (16) and cultured for 5 days in the absence or presence of DNP-EA, DNP-BGG, or EA. Under the conditions employed for depleting spleen cells of A cells, it was found that an average of $7.3 \times 10^5$ out of $2.2 \times 10^7$ (3.3%) spleen cells were adherent, and of these A cells, an average 80% of them were phagocytic (17). The NA cell population was found to contain less than 0.1% phagocytic cells.

In two separate experiments, whose results were averaged and are shown in Fig. 5, it was found that anti-DNP IgE production by NA cells was essentially the same as that by the whole spleen cell population. Maximum IgE production was observed in cultures that received no exogenous antigen, and both DNP-EA and DNP-BGG but not EA inhibited antihapten IgE production in NA cell cultures.

**Secondary Antihapten IgM and IgG Responses In Vitro.** Results of the preceding experiments all pointed to a direct effect by hapten on the IgE antibody-producing B cells. The next experiments were designed to examine the production of other classes of antihapten antibody under conditions of secondary antigen stimulation in vitro.

The results shown in Fig. 6 represent the secondary anti-DNP IgM PFC response after 5 days in vitro by spleen cells from mice primed 11 days earlier
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Fig. 6. Secondary anti-DNP PFC response in vitro. Spleen cells from CBA/J mice primed 11 days earlier with 1 μg DNP-EA + Al(OH)₃ gel (○-○) or from nonimmune control mice (●-●) were cultured for 5 days in the presence of DNP-EA (panel A), DNP-BGG (panel B), or EA (panel C). The number of direct (IgM) PFCs per 10⁸ viable lymphocytes was determined for duplicate cultures assayed in duplicate. The results shown represent the ± SEM of two separate experiments.

with 1 μg DNP-EA in 1 mg Al(OH)₃ gel. Also shown for purposes of comparison are the primary anti-DNP PFC response by spleen cells from nonimmune mice. Significant secondary IgM PFC responses were obtained when primed spleen cells were cultured in vitro with homologous hapten-carrier (DNP-EA) or carrier (EA) alone, but not when cultured in the presence of hapten coupled to heterologous carrier (DNP-BGG). In contrast to these results, DNP-BGG as well as DNP-EA (but not EA alone) stimulated a primary hapten-specific IgM PFC response by nonprimed splenic lymphocytes in vitro. The anti-DNP IgE antibody production and inhibition of production by primed spleen cells was essentially identical to that of previous experiments (Fig. 5) and is therefore not shown.

The DNP-EA primed lymphocytes in this experiment did not produce IgG PFCs, as determined by the indirect PFC assay (21). Subsequently, splenic lymphocytes were harvested from mice 21 days after primary immunization with 1 μg DNP-EA in Al(OH)₃ gel. The anti-DNP IgM and IgG PFC responses by the spleen cells cultured for 5 days in vitro are shown in Fig. 7, and the anti-DNP IgE antibody responses by the cultures are shown in Fig. 8.

The results shown in Fig. 7 indicate that the secondary antihapten IgG antibody response was enhanced by homologous hapten-carrier (DNP-EA) and carrier (EA) alone. In addition, the IgG response was not enhanced by hapten
Fig. 7. Secondary anti-DNP IgM and IgG PFC responses in vitro. Spleen cells from mice immunized 21 days earlier with 1 μg DNP-EA + Al(OH)₃ gel were cultured for 5 days, and the numbers of IgM (○--○) and IgG (●--●) PFCs per 10⁸ viable lymphocytes were determined. The number of IgG PFCs was calculated by subtracting the number of direct PFCs from the number of indirect (facilitated by incubating with rabbit antimouse IgG) PFCs. The results shown represent the x (± SEM) PFC responses of duplicate cultures assayed in duplicate, from two different experiments.

coupled to heterologous carrier, confirming the carrier-related specificity of the secondary IgG response in vitro.

The anti-DNP IgE responses by spleen cell cultures from mice primed 21 days in vivo, on the other hand, were suppressed by hapten, whether on homologous (DNP-EA) or heterologous (DNP-BGG) carrier (Fig. 8).

Discussion

The results of the present studies reveal that primed mouse IgE B cells are suppressed by antigen under conditions of in vitro exposure. The in vivo priming of mice induced a population of splenic lymphocytes which produced specific IgE antibody after 4–5 days of in vitro culturing. The addition of minute amounts of antigen (10⁻¹–10⁻¹ μg) to the lymphocyte cultures resulted in suppression of such "spontaneous" IgE production. The suppression was found not to be the result of neutralization of antibody by antigen.
Antigen-induced regulation of the reaginic antibody response in mice has been shown or postulated to occur in two ways: (a) directly at the B-cell level (2, 7, 26), and (b) indirectly, through helper or suppressor T cells (3-5, 10). By analyzing the anti-DNP IgE response by the primed lymphocytes to secondary stimulation by hapten coupled to homologous carrier (DNP-EA), hapten coupled to heterologous carrier (DNP-BGG), and carrier (EA) alone, we demonstrated that the antigen-induced inhibition of IgE production was caused by hapten and not by carrier. Our results suggest that the suppression by hapten of the in vitro IgE production was the result of a direct B-cell effect.

Production of IgE antibodies in vivo has been shown to be sensitive to T-cell regulation (3, 10, 27), and therefore it was decided to examine the role of T cells in the production or suppression of anti-DNP IgE in our experimental system. Neither the spontaneous in vitro production of anti-DNP IgE nor the hapten-induced suppression of such antibody production was affected by depleting spleen cell cultures of T cells by treatment with anti-θ plus C'. Thus, T cells were not required for the maximum production of anti-DNP IgE by spleen cells primed 11 days earlier in vivo. IgE production in vitro by B cells at this stage of differentiation has not previously been studied by others. Okudaira and Ishizaka (28) reported that IgE B cells tend to lose their T-cell dependency if placed in tissue culture for 24-48 h before transfer into irradiated recipient mice, and it is possible that the milieu afforded by our tissue culture conditions was similar to that offered by an irradiated recipient.

Macrophages (A cells) have been shown by others to be involved in antigen-induced suppression of immune responses (24, 25, 29), and therefore the possibility that these cells were involved in the hapten-induced suppression of IgE was examined. The depletion of primed spleen cultures of A cells was
found to have no effect on the production of, or on the hapten-induced suppression of, anti-DNP IgE in vitro. Ishizaka and Kishimoto (30), using mesenteric lymph node cells from antigen-primed rabbits and culturing them in vitro, also reported that A cells had no effect on IgE antibody production in vitro.

Although it is not certain that all macrophages or T cells had been removed from the spleen cell cultures, a drastic reduction in the numbers of these cell populations was accomplished and this did not affect the suppressive effects of hapten on IgE production. These results, taken together with the lack of carrier effect, lead us to conclude that the hapten was interacting directly at the level of the IgE-producing B cell, resulting in a state of nonresponsiveness. Such IgE B-cell tolerance in mice in vivo has been reported by Lee and Sehon (6–8) using DNP coupled to mouse γ-globulin (DNP-MγG). Although in their system, tolerance was presumably due to the lack of carrier-specific T cells, they found that anti-DNP IgE B cells were made tolerant more easily (i.e., at lower doses of DNP-MγG) than anti-DNP IgG B cells. Lee and Sehon (7) speculated that the hapten-specific tolerance induced in mice by treatment with DNP-MγG involved the elimination or inactivation of hapten-specific IgE B cells, or to the blockade of the receptors of IgE B cells. A similar mechanism seems to be operating in the hapten-induced suppression of anti-DNP IgE production in vitro reported in the present study, even though an immunogenic carrier (EA) was used.

Under the priming and tissue culture conditions of our experiments, it was found that the optimal concentrations of antigen required for secondary responses in vitro were similar for the IgM and IgG classes of antibody. Concentrations optimal for IgG and IgM responses caused suppression of the IgE response. Carrier alone enhanced antihapten IgM and IgG antibody responses in vitro, confirming the reports of Kimoto et al. (31) and Kishimoto and Ishizaka (32). In our studies, carrier alone neither enhanced nor suppressed the IgE response. Our priming regimen, which was required for in vitro IgE antibody formation, differed from that employed by Kimoto et al. (31) who used spleen cell cultures from mice primed with three doses of antigen (1 μg) injected at 4-wk intervals. It can be assumed that Kimoto et al. established a true anamnestic IgE response in vitro, which was subject to carrier (T cell) enhancement or suppression. In our study, in which cultures were established 11 days after a single primary immunization, either the IgE B cells were refractory to T-cell signals or T cells present in our cultures were not present in high enough numbers or did not possess the capacity to suppress or enhance the production of IgE by B cells in vitro.

It has been shown that antibody specific for an antigen can regulate further production of that antibody. This process, called antibody-mediated feedback inhibition (33), was postulated by Tada and Okumura (34, 35) to be responsible for the 7S antibody-induced inhibition of hapten-specific IgE antibody production by rats in vivo (34). Ishizaka and Okudaira (36), however, reported that anti-EA IgG antibody failed to depress anti-EA IgE antibody responses in mice. In the in vitro experiments reported here, endogenous production of one class of antibody (e.g., IgM or IgG) did not seem to play a role in the regulation of the IgE class of antibody. The fact that EA alone enhanced anti-DNP IgM/
IgG immune responses in vitro but had no detectable effect on the spontaneous anti-DNP IgE response, and the fact that DNP-BGG had no effect on the anti-DNP IgM/IgG secondary immune response in vitro but suppressed anti-DNP IgE production, suggest that the IgE antibody response in vitro was not regulated by endogenous production of anti-DNP antibodies of other Ig classes.

Summary

Regulation of IgE production by antigen in a primed murine splenic lymphocyte culture system was described. Maximum IgE antibody production was found to occur when cells were cultured in the absence of exogenously added antigen. A cells and T lymphocytes did not affect the production of anti-DNP IgE antibody.

By using a hapten-carrier antigen system (DNP-EA) for priming mice in vivo, it was found that the production of anti-DNP IgE by spleen cells in vitro was inhibited by hapten when coupled to homologous (EA) or heterologous (BGG) carrier, and was not enhanced or inhibited by homologous carrier. Anti-DNP IgE antibody production by cultures depleted of macrophages or T lymphocytes was found to be as sensitive to the suppressive effects of hapten as was the IgE production by whole spleen cell cultures.

Both IgM and IgG secondary anti-DNP PFC responses in vitro were enhanced by the presence of the homologous hapten-carrier or carrier alone. DNP-BGG had no effect on the anti-DNP IgM or IgG PFC responses of the cultures. These data suggest that endogenous production of antibody (IgM or IgG) was not responsible for the observed suppression of the IgE response in vitro.

The experimental results presented indicate that the regulation of the IgE production by antigen in the primed mouse splenic lymphocyte cultures was a consequence of the direct interaction of hapten with IgE B cells.

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