INHIBITION OF AN IgE RESPONSE BY SECONDARY B CELLS OF A DIFFERENT ISOTYPE

BY SEIJI HABA, MICHAEL F. GURISH, AND ALFRED NISONOFF

From the Rosenstiel Research Center, Department of Biology, Brandeis University, Waltham, Massachusetts 02254

Under certain circumstances, secondary B cells can preempt an immune response and inhibit the expression of the products of primary B cells having the same specificity. This effect was illustrated by experiments (1) that made use of a major intrastrain crossreactive idiotype characteristic of the anti-Ars antibodies of strain A mice (CRI\textsubscript{A}). The idiotype is absent in the anti-Ars antibodies of BALB/c mice (H-2\textsuperscript{d}; Igh\textsuperscript{a}), but present in allotype-congenic C.AL-20 mice (H-2\textsuperscript{d}; Igh\textsuperscript{d}; references 1, 2). After transfer of lymphocytes from Ars-immune BALB/c mice to mildly irradiated (200 rad) nonimmune C.AL-20 recipients, the latter produced no CRI\textsubscript{A}\textsuperscript{*} antibodies after immunization with KLH-Ars. It was shown (1) that the inhibition of CRI\textsubscript{A} expression was mediated by the B cells of the donor and that T cells were ineffective. A possible synergistic effect of small amounts of contaminating carrier-specific Ts cells was ruled out by using different carriers for the hapten in the donor and recipient mice. The inhibition was shown to persist for at least 5 mo after an adoptive transfer. Similar experiments were carried out (3) by using A/J mice as a source of lymphoid cells for adoptive transfer. These donor mice were idiotypically suppressed for CRI\textsubscript{A} and then immunized. Mildly irradiated A/J mice that received either enriched B or T cells from such donors expressed little or no CRI\textsubscript{A} upon subsequent immunization against the Ars hapten. On a numerical basis, B cells were somewhat more suppressive than T cells.

Experiments of similar design indicated that this effect can be used to suppress an allotype as well as an idiotype (4). Spleen cells from BALB/c mice immunized with KLH-TNP were adoptively transferred into irradiated (200 rad) allotype-congenic partners (C.B-17), which were then immunized to TNP. Through day 58 after the adoptive transfer, $\geq$80% of the IgG antibody produced was of donor allotype. By day 110, the allotype of the host dominated in most but not all recipients. Other recipients that were given nonimmune BALB/c cells produced antibodies almost entirely of the recipients’ allotype. The participation of T cells in the suppression was not completely ruled out but appears improbable; i.e., it seems unlikely that immunized BALB/c mice would produce Ts cells specific for an unrelated allotype. An explanation consistent with each set of results discussed

This work was supported by National Institutes of Health grants AI-22068 and AI-24272. M. F. Gurish was supported by a postdoctoral fellowship from the Arthritis Foundation.

Abbreviation used in this paper: CRI\textsubscript{A}, a major intrastrain crossreactive idiotype characteristic of the anti-Ars antibodies of strain A mice.
above is that secondary B cells can dominate a response by virtue of their great numerical superiority and greater ease of triggering as compared with the primary B cells of the same specificity, present in the recipient animal.

The present experiments were designed to ascertain whether this effect can be exploited to inhibit the synthesis of IgE antibodies of a given specificity. The experiments made use of the observation that immunization with antigen emulsified in CFA induces the synthesis of large amounts of antibodies that are almost devoid of IgE. This permitted the adoptive transfer of non-IgE-producing immune B cells into naive recipients, which were then tested for their capacity to produce IgE antibodies of the same specificity. Related experiments were carried out without adoptive transfer by immunizing individual mice successively with antigens in CFA and alum; the latter adjuvant is permissive for IgE synthesis.

Materials and Methods

Mice. Female A/J mice were obtained from The Jackson Laboratory, (Bar Harbor, ME). Within an experiment, the mice were age matched. The range of ages at the start of an experiment was 6–12 wk.

Antigens and Adjuvants. KLH was obtained from Calbiochem-Behring Corp. (La Jolla, CA); OVA was from Miles Laboratories Inc. (Naperville, IL); and BSA (fraction V) was from Sigma Chemical Co. (St. Louis, MO). Ars derivatives of KLH, OVA, or BSA were prepared by diazotization (5). TNP derivatives of KLH and BSA were prepared by conjugating trinitrobenzene sulfonate to the protein at pH 8.0 in saline-borate buffer for 2 h at room temperature (6). The weight ratio of hapten to protein used for coupling was 1:25. Alum was prepared from aluminum sulfate and sodium hydroxide according to the method of Levine and Vaz (7). The alum was thoroughly mixed with antigen solution shortly before use; 4 mg of alum was present in each inoculum, and the volume injected was 0.5 ml. CFA (Difco Laboratories Inc., Detroit, MI) was used as a 1:1 emulsion with the antigen solution or with saline; 0.2 ml of the emulsion was inoculated into each mouse. All inoculations were given intraperitoneally. Bleedings were taken 6 d after the second and third inoculations of antigen in alum. We found that IgE anti-Ars titers are at a maximum 6 d after challenge.

Radioimmunoassays. Assays for total anti-Ars were carried out on microtiter plates whose wells were coated with BSA-Ars, using a 1 mg/ml solution of the conjugate (8, 9). We used 125I-labeled, affinity-purified rabbit anti-mouse Fab as the developing reagent. Assays for IgE anti-Ars were done in a similar manner, using 125I-labeled affinity-purified rabbit anti-mouse IgE for development (10). The anti-IgE was prepared against the IgE protein TIB-142 (American Type Culture Collection, Rockville, MD; donated by M. Wabl), which has anti-TNP activity. Adsorptions of the anti-IgE were carried out as described elsewhere (11). For affinity purification, the mAb SE1.3 (anti-Ars, IgE; reference 12) was conjugated to Sepharose-4B; after passage through a column of this adsorbent, anti-IgE was eluted with 3 M NaSCN.

To minimize errors owing to the presence in sera of non-IgE anti-Ars (which competes with IgE for binding to the BSA-Ars on the microtiter plate), serum samples were precipitated with ammonium sulfate, at 39% of saturation, before the assay for IgE anti-Ars. This removes a large proportion of IgG anti-Ars while leaving 80–100% of the IgE in solution (11). Despite the precipitation step, a few samples required moderate corrections, up to 25%, because of the presence of residual non-IgE anti-Ars. The procedure for making such corrections, which requires a prior determination of total anti-Ars, is described in detail in reference 11. Assays for total anti-TNP and IgE anti-TNP antibodies were carried out by procedures analogous to those used for total and IgE anti-Ars. Wells were coated with BSA-TNP (1 mg/ml). All assays were carried out in duplicate. The data in the tables are expressed as mean values for the group of mice tested ± SEM.

Standards used for the assays for total anti-Ars and IgE anti-Ars were anti-Ars antibodies
affinity purified from hyperimmune A/J ascites (13) and the mAb SE20.2 (anti-Ars, IgE; reference 12), respectively. The standard used for total anti-TNP was a DEAE-cellulose-purified fraction of ascitic fluids of KLH-TNP-immune A/J mice. The anti-TNP content was determined by the quantitative precipitin test. Precipitates were dissolved in 0.1 M NaOH and absorbances at 280 and 400 nm were determined. The latter value was used to correct for the content of antigen. The standard for IgE anti-TNP was mAb TIB-142, affinity purified as described elsewhere (11).

Preparation of Enriched Spleenic T and B Cells. Single-cell suspensions of spleen were treated on ice for 5 min with a solution containing 0.155 M NH₄Cl, 0.01 M KHCO₃, and 0.001 M EDTA, pH 7.4, to lyse erythrocytes. To enrich for T cells, we used two cycles of panning on anti-Ig antibody–coated plastic surfaces (14). In brief, leukocytes in RPMI 1640 medium, supplemented with 5% FCS, were placed on 100-mm Petri dishes (10⁶ cells/5 ml medium/dish) precoated with a mixture of affinity-purified rabbit anti-mouse Fab antibodies (10 μg/ml) and nonspecific rabbit IgG (90 μg/ml). The cells were allowed to stand for 40 min at 4°C, and after gentle agitation, for an additional 30 min (1st cycle). The nonadherent cells were resuspended by swirling and were then removed. The plates were washed twice with cold PBS containing 3% FCS, and the supernatant was added to the pool of nonadherent cells. The nonadherent cells (6 × 10⁷/5 ml medium/dish) were subjected to a second cycle of panning in the same manner, on dishes precoated with 200 μg/ml (rather than 10 μg/ml) of affinity-purified rabbit anti-mouse Fab antibodies. Recovery of the nonadherent cells (T cell fraction) was 25–30% of the original spleen cell population; >80% of these cells were killed by monoclonal anti-Thy-1.2 plus C.

To enrich for B cells, the adherent cells were recovered from the anti-Fab–coated plates used for the first cycle of panning. The plates were washed three times with cold PBS. 10 ml of PBS containing 3% FCS was added at room temperature, and adherent cells were released by vigorous pipeting, using a 10-ml plastic pipet. This cell population was then treated with anti-Thy-1.2 and “Low-Tox” rabbit complement (Cedarlane Laboratories, Hicksville, NY). Recovery of this enriched B cell population was ~25% of the original cell population, and >95% of the cells were viable. A second treatment of these cells with anti-Thy-1.2 plus C showed the absence of significant contamination by T cells.

Results

The major aim of this work was to determine whether IgE anti-Ars synthesis is inhibited by the presence of B cells committed to the synthesis of non-IgE anti-Ars. Our approach to this question made use of previous observations (15–18), which indicated that antigens administered in CFA yield high titers of antibodies containing little if any IgE. In general, alum is a much better adjuvant for the induction of IgE antibodies (e. g., reference 18). Our first experiments were carried out to test these premises and to establish conditions appropriate for investigating the inhibition of IgE synthesis by non-IgE-producing B cells.

The data in Table 1, groups 1 and 2, indicate that alum is much more effective than CFA as an adjuvant for the production of IgE anti-Ars antibodies. For example, at day 48, when the titers of total anti-Ars were comparable in the two groups, more than 20 times as much IgE anti-Ars was present in mice immunized with KLH-Ars in alum.

In addition, the data for groups 3 and 4 (compared with group 1) indicate that the prior administration of KLH-Ars in CFA greatly inhibited the production of IgE anti-Ars (but not total anti-Ars), when the mice were subsequently challenged with KLH-Ars in alum. The results for groups 1 and 5 demonstrate that similar inhibition of IgE production was induced by prior administration, in CFA, of unconjugated KLH; i. e., the presence of hapten groups in the immunogen was not required to cause inhibition of IgE anti-Ars production.
In contrast, preinoculation of CFA alone (group 6) did not result in selective inhibition of IgE anti-Ars formation. On day 27, the titer of IgE anti-Ars was somewhat lower than that of group 1, but the ratios of IgE/total anti-Ars are similar in the two groups. On day 48, this ratio is actually somewhat higher in mice of group 6, which had received CFA; this is due to a lower total anti-Ars titer in group 6, since the IgE anti-Ars concentrations are similar.

Mice in group 7 received four inoculations of CFA without antigen before challenge with KLH-Ars. This appears to result in considerable inhibition of total anti-Ars titers obtained after challenge with KLH-Ars in alum, but in either no effect (day 27) or an enhancement (day 48) of IgE anti-Ars antibody titers.

The experiments described in Table II served two purposes: to ascertain the effect of preinoculation of a higher dose (100 vs. 20 μg) of KLH-Ars or KLH in CFA, and to test the effects of preinoculation, in CFA, of another pair of antigens, OVA and OVA-Ars. In all cases, mice were subsequently challenged three times, with KLH-Ars in alum, on days 0, 21, and 42.

Results obtained for group 2 (Table II) confirmed the absence of any significant inhibitory effect of preinoculation of CFA alone on the subsequent IgE anti-Ars response to KLH-Ars in alum; we saw a moderate decrease on day 27, but the two groups were very similar on day 48. The inhibitory effect of preinoculation of CFA on the total anti-Ars response (Table I) was confirmed by results obtained for group 2 (Table II).

The data in Table II, groups 3 and 4, further demonstrate that preinoculation of a high dose (100 μg) of either KLH-Ars or KLH markedly inhibited the subsequent IgE anti-Ars response to KLH-Ars administered in alum (cf., groups 1, 2, 3, and 4).

Results for groups 5 and 6, however, indicate a marked difference between the effects of preinoculation of OVA and OVA-Ars. There was strong inhibition

---

**TABLE I**

*Effects of Preinoculation of CFA, with or without Antigen, on Stimulation of IgE anti-Ars by KLH-Ars in Alum*

| Group | Antigen       | Days of inoculations | Day 27 | Day 48 |
|-------|---------------|----------------------|--------|--------|
|       |               |                      | Total $\times 10^3$ IgE | Total $\times 10^3$ IgE | Ratio of IgE/total anti-Ars $\times 10^4$ |
| 1     | KLH-Ars (Alum)| 0, 21, 42            | 240 ± 70 | 2,070 ± 640 | 3,380 ± 1,000 | 2,280 ± 330 | 6.7 |
| 2     | KLH-Ars (CFA) | 0, 21, 42            | 1,980 ± 580 | 20 ± 10 | 4,540 ± 1,060 | 100 ± 50 | 0.2 |
| 3     | KLH-Ars (CFA) | 42, 21               | 10,970 ± 1,240 | 40 ± 40 | — | — | — |
| 4     | KLH-Ars (CFA) | 0, 21, 42            | 1,650 ± 460 | 330 ± 120 | 7,750 ± 890 | 190 ± 120 | 0.2 |
| 5     | KLH-Ars (Alum)| 0, 21, 42            | 240 ± 120 | 320 ± 150 | 1,800 ± 78 | 280 ± 140 | 1.5 |
| 6     | CFA (saline)  | 0, 21                 | 110 ± 40 | 990 ± 410 | 810 ± 170 | 2,740 ± 870 | 53.8 |
| 7     | KLH-Ars (Alum)| 0, 21, 42            | 110 ± 40 | 2,090 ± 810 | 390 ± 80 | 6,280 ± 1,630 | 161 |

Each group contained seven mice. Results are mean values ± SEM. Inoculations were intraperitoneal, using 20 μg of antigen (with CFA) or 5 μg (with alum). The volume ratio of antigen solution (or saline) to CFA was 1:1 and 0.2 ml was inoculated. 4 μg of alum was used and the final volume was 0.5 ml.
Effect of Preinoculations of Antigens on the Total and IgE Anti-Ars Response to KLH-Ars in Alum

| Group | Preinoculations | Anti-Ars Titers (ng/ml) |
|-------|----------------|-------------------------|
|       |                | Day -15 | Day -1 | Day 27 | Day 48 | Ratio of IgE/Total Anti-Ars |
|       |                | Total | IgE | Total | IgE | Total | IgE | Ratio |
|-------|----------------|-------|-----|-------|-----|-------|-----|-------|
| 1     | None           |       |     |       |     |       |     |       |
| 2     | CFA            |       |     |       |     |       |     |       |
| 3     | 100 µg KLH-Ars in CFA | ± 210 | ± 20 | ± 540 | ± 30 | ± 60 | ± 40 | ± 980 |
| 4     | 100 µg KLH in CFA |       |     |       |     |       |     |       |
| 5     | 200 µg OVA-Ars in CFA | ± 140 | ± 2 | ± 290 | ± 10 | ± 40 | ± 220 | ± 110 |
| 6     | 200 µg OVA in CFA |       |     |       |     |       |     |       |

Each group contained 9 (groups 2, 5, 6) or 10 mice (groups 1, 3, 4). The preinoculations specified were given on days -42 and -21; inoculations of KLH-Ars in alum (5 µg) were given on days -1, 0, 21, and 35. All inoculations were intraperitoneal (see Table I for volumes inoculated).

* Probability of a significant decrease in the IgE anti-Ars titer as compared with group 2. Statistical analyses were carried out by the Student's t-test.

of the synthesis of IgE anti-Ars after preinoculation of OVA-Ars in CFA, but we saw little if any effect when unconjugated OVA was administered in CFA before challenge with KLH-Ars in alum.

Adoptive Transfer of Spleen Cells or Enriched T or B cells. To explore further the mechanism of the inhibition of IgE anti-Ars synthesis that was induced by preinoculation of KLH, KLH-Ars, or OVA-Ars (but not OVA), adoptive transfer experiments were carried out (Table III). Donor A/J mice were either not immunized, immunized with CFA alone, or with antigen in CFA. 3 $\times$ 10$^7$ cells were transferred (day -1) into normal adult A/J recipients, which were then immunized intraperitoneally with 5 µg KLH-Ars in alum on days 0, 21, and 35. Control mice (groups 1–7) received no cells, cells from naive animals, or cells from animals that had received CFA without antigen.

It is evident, first, that adoptive transfer of spleen cells, or enriched T or B cells from mice immunized with CFA (groups 5–7), had little effect on the IgE response at day 41. This confirms the results obtained by preinoculation of CFA, followed by KLH-Ars in alum (Tables I and II). In contrast, marked selective inhibition of IgE anti-Ars synthesis was noted after adoptive transfer of spleen cells or B cells from mice immunized with KLH-Ars in CFA (groups 8–10; see $p$ values). After adoptive transfer of enriched T cells (group 9), there was some inhibition of total, as well as IgE anti-Ars synthesis, possibly reflecting the presence of a greater concentration of Ts cells in this population, as compared with unfractionated spleen cells.

Adoptive transfer of cells from mice treated with KLH (rather than KLH-Ars) in CFA failed to confirm the selective inhibition of IgE anti-Ars noted (Tables I and II) after preinoculation of KLH without adoptive transfer. There was little if any inhibition of IgE anti-Ars synthesis (cf., groups 5–7 with groups 11–13).

A point of major interest in Table III emerges from the effects of transferring
cells from donors immunized with OVA-Ars or OVA. OVA-Ars-immune spleen cells or enriched B cells caused a marked selective inhibition of IgE anti-Ars synthesis (groups 14 and 16), whereas we saw no such effect after transfer of OVA-Ars-immune T cells (group 15). None of the transferred cell populations from donors immunized with unconjugated OVA were significantly inhibitory with respect to IgE anti-Ars production (see $p$ values, groups 17–19).

We next examined the dose response of mice receiving varying numbers of cells (on day −1) from donors that had been immunized with OVA-Ars in CFA before the adoptive transfer. Recipients were challenged with KLH-Ars in alum on days 0, 21, and 35. The data for groups 8 and 9, Table IV, confirm the

### Table III

| Group | Immunization of donors | Cells transferred | Day 15 | Day 27 | Day 41 |
|-------|------------------------|------------------|--------|--------|--------|
|       |                        |                  | Total IgE | Total IgE | Total IgE |
|       |                        |                  | $x 10^6$ | $x 10^6$ | $x 10^6$ |
| 1     | Nonimmune Spleen       | 0                | 1,280   | 2,670   | 2,200   |
| 2     | Nonimmune T            | $\pm 20$         | $\pm 40$ | $\pm 50$ | $\pm 50$ |
| 3     | Nonimmune B            | $\pm 3$          | $\pm 140$ | $\pm 150$ | $\pm 150$ |
| 4     | CFA Spleen             | 0                | 200     | 600     | 2,000   |
| 5     | CFA T                  | 0,60             | 670     | 1,270   | 5,390   |
| 6     | CFA B                  | 0,80             | 90      | 120     | 1,990   |
| 7     | KLH-Ars (CFA) Spleen   | 0                | 1,280   | 2,670   | 2,200   |
| 8     | KLH-Ars (CFA) T        | 0                | 200     | 600     | 2,000   |
| 9     | KLH-Ars (CFA) B        | 0                | 200     | 600     | 2,000   |
| 10    | KLH (CFA) Spleen       | 0                | 200     | 600     | 2,000   |
| 11    | KLH (CFA) T            | 0                | 200     | 600     | 2,000   |
| 12    | KLH (CFA) B            | 0                | 200     | 600     | 2,000   |
| 13    | OVA-Ars (CFA) Spleen   | 0                | 200     | 600     | 2,000   |
| 14    | OVA-Ars (CFA) T        | 0                | 200     | 600     | 2,000   |
| 15    | OVA-Ars (CFA) B        | 0                | 200     | 600     | 2,000   |
| 16    | OVA (CFA) Spleen       | 0                | 200     | 600     | 2,000   |
| 17    | OVA (CFA) T            | 0                | 200     | 600     | 2,000   |
| 18    | OVA (CFA) B            | 0                | 200     | 600     | 2,000   |
| 19    | KLH (CFA) Spleen       | 0                | 200     | 600     | 2,000   |

Donors were immunized on days −49 and −28 with 100 μg of the protein antigen in CFA, or with CFA emulsified with saline. $5 \times 10^6$ cells were transferred intravenously into naive recipients on day −1. Recipients were immunized intraperitoneally with 5 μg KLH-Ars in alum on days 0, 21, and 35. Groups 8 and 9 contained six mice, other groups, seven mice.

* For a decrease in the IgE anti-Ars titer as compared with the group number specified in parentheses.
absence of any significant inhibitory effect of transferred T cells on subsequent IgE anti-Ars production. In contrast, 3 \times 10^7 or 1 \times 10^7 unfractionated spleen cells caused a highly significant decrease in IgE anti-Ars production, whereas 3 \times 10^6 spleen cells had considerably less effect (groups 1–4). Very similar results were obtained after transfer of enriched B cells; i.e., marked inhibition of IgE anti-Ars after transfer of 3 \times 10^7 or 1 \times 10^7 cells, and a lesser effect of 3 \times 10^6 cells (groups 1, 5–7). These results indicate that the inhibition of IgE anti-Ars synthesis by B cells is not attributable to contamination with T cells.

The specificity of inhibition of IgE synthesis after adoptive transfer of OVA-Ars-primed spleen cells is shown by the data in Table V. Again, marked inhibition of IgE anti-Ars synthesis is seen in the mice that received spleen cells from donors immunized with OVA-Ars in CFA (group 2), but not in mice that received OVA/CFA-primed spleen cells (group 3). When the recipients of OVA-Ars-primed cells were immunized with KLH-TNP, we saw no significant decrease in IgE anti-TNP (cf., groups 4 and 5).

### Discussion

The results reported here bear on the following subjects: (a) inhibition of antibodies of a given isotype (IgE) mediated by secondary B cells producing antibodies of the same specificity but of a different isotype; (b) other effects on IgE synthesis of preinoculations of CFA, or CFA plus antigen, before challenge with antigen in alum. The experiments were based on the previous observations (15–18) that minimal or undetectable amounts of IgE antibodies are produced when certain antigens are administered to mice (or rats) as an emulsion in CFA,
although high concentrations of non-IgE antibodies are induced. Our experiments (Tables I and II) confirmed these reports. This permitted adoptive transfer experiments to identify the cell type(s) responsible for the inhibition of IgE production. Because several parameters need to be discussed we will consider the data in terms of the antigen used for preinoculation (when individual mice were immunized successively with two substances), or for immunizing donors before adoptive transfer.

**Preinoculations of OVA or OVA-Ars in CFA.** OVA and OVA-Ars will be considered first because they appear to provide the most cogent data supporting an inhibitory effect of antigen-specific non-IgE B cells on the synthesis of IgE antibodies of the same specificity. When mice were inoculated with OVA-Ars in CFA before KLH-Ars in alum, there was marked inhibition of the IgE anti-Ars response as compared with mice that received only CFA before KLH-Ars (Table II). There was no significant inhibition of the total anti-Ars response. Preinoculation of OVA, rather than OVA-Ars in CFA, did not inhibit IgE (or total) anti-Ars synthesis upon subsequent challenge with KLH-Ars in alum. Adoptive transfer experiments (Table III) indicated that the inhibitory effect on IgE anti-Ars synthesis of preinoculation of OVA-Ars in CFA is mediated by B cells. Unfractionated spleen cells or enriched B cells were inhibitory in recipient mice, whereas the same number (3 x 10^7) of enriched T cells was not. Cells transferred from OVA/CFA-immune mice had no significant effect on IgE anti-Ars production. The experiments using OVA-Ars followed by KLH-Ars seem particularly informative, since the change of carrier protein eliminates effects that might be attributable to carrier-specific suppressor cells.

Further evidence that the inhibitory effect on IgE antibody synthesis, after adoptive transfer, is mediated by B cells are the data on dose response. Whereas 10^7 enriched B cells from OVA-Ars/CFA-immune mice caused significant inhibition, 3 x 10^7 enriched T cells were noninhibitory. This appears to rule out contamination by T cells as an explanation for the B cell effect. The specificity of the phenomenon was indicated by the failure of transferred Ars-immune...
spleen cells to affect anti-TNP IgE synthesis. Possible mechanisms for the
inhibition by B cells are discussed below.

Preinoculation of KLH-Ars/CFA. These data are consistent with an inhibitory
effect of Ars-specific, non-IgE B cells on IgE anti-Ars synthesis; they also bear
on the effect of T cells. Preinoculation of KLH-Ars/CFA caused a highly
significant decrease in IgE anti-Ars synthesis upon challenge with KLH-Ars in
alum (Table II). In contrast to preinoculation of OVA-Ars, this inhibitory effect
was noted after adoptive transfer of enriched T cells, as well as B cells or spleen
cells (Table III). This suggests a possible inhibitory role for carrier-specific (19–
22) or hapten-specific (23) Ts cells. This possibility is supported by the moderate
decrease in total anti-Ars (p = 0.024), as well as IgE anti-Ars synthesis (p =
0.033) after adoptive transfer of KLH-Ars-immune T cells (Table III, group 9
vs. group 6).

Preinoculation of KLH/CFA. The effect of preinoculation of KLH in CFA
before challenge with KLH-Ars in alum is consistent with a carrier-specific T
cell effect. There was a highly significant decrease in IgE anti-Ars synthesis
(Table II, cf., groups 2 and 4). Since hapten was not conjugated to the antigen
used for preinoculation, it is difficult to invoke a B cell effect. In this case,
adoptive transfer experiments were not consistent with the results of the prein-
oculations because the transfer of KLH/CFA-immune spleen, T or B cells had
little effect on the subsequent response to KLH-Ar in alum. It is not obvious
why the adoptive transfer experiments were negative, whereas preinoculation of
KLH within an individual mouse was inhibitory. A possible explanation is that
the effect of T cells is simply diminished in this case by the adoptive transfer, as
compared with their effect in the intact mouse.

Preinoculation of CFA. Inoculations of CFA alone, twice or four times, before
challenge with KLH-Ars in alum had no inhibitory effect on IgE anti-Ars titers
(Table I). There was, however, a definite inhibitory effect on total anti-Ars titers,
particularly after four preinoculations of CFA; i. e., four inoculations of CFA
increased the ratio of IgE/total anti-Ars. The absence of an effect of CFA
injection on IgE anti-Ars titers is supported by the results of adoptive transfers
of splenic T or B cells (Table III). The effect on total anti-Ars titers, after the
adoptive transfers, was also not significant (p > 0.2 in each case; p values for
total anti-Ars not shown in the Table). Thus, our data showed no significant
effect on IgE production of preinoculations with CFA without antigen. This
appears to contrast with some (but not all) of the data in the literature. However,
the regimen of inoculations and the strain or species used have varied consider-
ably. For example, Tung et al. (24) found that irradiated CAF1 mice that received
50 × 10⁶ spleen cells from syngeneic donors that had been primed with a DNP-
conjugated extract of ascaris, produced high titers of IgE anti-DNP antibodies
after immunization with the same antigen. Pretreatment of the recipients with
CFA virtually eliminated the IgE response. They also showed that CFA treatment
of low or intermediate responder mice (B6 or AKR, respectively) induced a
serum factor that inhibited the IgE response in lightly irradiated syngeneic
recipients. This effect was not seen in two high responder strains: BALB/c and
(SJL× BALB/c)F₁.

In other studies it was shown that the serum or ascites of CFA-treated mice
contain factors that can enhance or suppress IgE responses (25). IgE suppressive factor has also been identified in supernatants of cultured lymphoid cells taken from CFA-primed rats (26) or from rats infected with Nippostrongylus brasiliensis (27). The latter treatment, however, resulted in the production of IgE-potentiating as well as IgE-suppressive factors (28).

A strong and selective inhibitory effect of preinoculation of CFA on IgE anti-OVA production was noted by Smith and Butchko (29) in both high- or moderate-responder mice. The difference in our results may be attributable to differences in timing of administration of CFA and antigen, which was shown to be a critical factor in one system investigated (30).

Possible Mechanisms of Inhibition of IgE Anti-Ars Production by Non-IgE anti-Ars-specific B cells. This is the major question addressed by our experiments. We have included a discussion of IgE suppression by T cells because some of our data are indicative of such an effect. Similar observations of inhibition by secondary B cells have been made in other systems, using idiotypic or allotypic markers. These experiments are described in the Introduction. One interpretation of such results (1, 3, 4) is that secondary B cells exercise their inhibitory effect through a mechanism of clonal dominance over primary B cells based on their large numerical superiority over the primary cells that differ with respect to idiotype or allotype; as a consequence, the secondary B cells should be far more effective in competing for antigen. Preferential triggering of secondary cells may also be reinforced by a higher density of surface Ia molecules (31), as well as a greater average avidity for the antigen (32, 33). Recent results of Takemori and Rajewsky (34) suggest, however, that B cells may also play a more active role in suppression; in their system, adoptively transferred, unprimed, idiotypically suppressed B cells prevented the expression of the idiotype by normal B cells. The mechanism of this effect is not known. The possibility must be considered that such a suppressive effect could also be operative at the level of isotype. The coexistence of IgG with small amounts of IgE antibodies in normally immunized animals implies that once the process of inducing IgE is initiated, the clonal dominance effect no longer can suppress IgE formation.

Antigen-specific suppression mediated by B cells is well documented. Possible explanations have included inhibition by secreted antibodies via blockade of epitopes of the antigen, or triggering of an antiidiotypic (antibody or T cells) response leading to antigen-specific suppression (35). Other investigators have observed the secretion of soluble suppressive factors by B cells exposed to antigen-antibody complexes (36) or to heat-aggregated Ig (37). These factors suppress polyclonal B cell responses, apparently by a nonspecific mechanism. The studies of Masuda and collaborators (38, 39) indicate that the factor is secreted by FcR+ B cells and that neither T cells nor macrophages are required. None of these observations appears to bear directly on the present results, in which we saw isotype-specific inhibition by secondary B cells.

If our interpretation of the data is correct, i.e., that inhibition is attributable to clonal dominance of secondary B cells, it might be possible to effect long-term inhibition of an IgE response through a manipulation that temporarily suppresses IgE synthesis, while at the same time stimulating non-IgE B cells of the same specificity.
We found that the synthesis of IgE anti-Ars antibodies is strongly inhibited by the presence of secondary non-IgE-producing cells that are specific for the Ars hapten. Such B cells can be induced by inoculation of a protein-Ars conjugate in CFA. The effect is seen after inoculation of OVA-Ars in CFA followed by KLH-Ars in alum, or, more convincingly, after adoptive transfer of B cells induced by antigen in CFA. Dose-response data indicated that inhibition can be effected by B cells containing noninhibitory numbers of contaminating T cells. Possible synergistic effects of carrier-specific regulatory T cells were ruled out by using a different protein carrier for immunization of donor and recipient mice. The effect was shown to be specific for the hapten used for immunization of donor mice.

References
1. Fig, B. M., S.-T. Ju, and A. Nisonoff. 1977. Complete inhibition of the expression of an idiotype by a mechanism of B cell dominance. J. Exp. Med. 146:1574.
2. Pawlak, L. L., E. B. Mushinski, and A. Nisonoff. 1973. Evidence for the linkage of the IgCh locus to a gene controlling the idiotypic specificity of anti-p-azophenylarsonate antibodies in strain A mice. J. Exp. Med. 137:22.
3. Ward, K., H. Cantor, and A. Nisonoff. 1978. Analysis of the cellular basis of idiotype-specific suppression. J. Immunol. 120:2016.
4. Brown, A. B., C. L. DeWitt, M. J. Bosma, and A. Nisonoff. 1980. Dominance of an immune response by secondary cells: quantitation by allotype analysis. J. Immunol. 124:250.
5. Nisonoff, A. 1967. Coupling of diazonium compounds to proteins. Methods Immunol. Immunochem. 1:120.
6. Mishell, B., and S. M. Shiigi. 1980. Selected Methods in Cellular Immunology. W. H. Freeman and Co., San Francisco, CA. 345 pp.
7. Levine, B. B., and N. M. Vaz. 1970. Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy. Int. Arch. Allergy Appl. Immunol. 39:156.
8. Klinman, N. R., A. R. Pickard, N. H. Sigal, P. J. Gearhart, F. S. Metcalf, and S. K. Pierce. 1976. Assessing B cell diversification by antigen receptor and precursor cell analysis. Ann. Immunol. (Paris). 127:489.
9. Dohi, Y., and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. J. Exp. Med. 150:909.
10. Haba, S., T. Inada, and A. Nisonoff. 1984. Quantitative measurements of an intrastrain cross-reactive idiotype in IgE antibodies. J. Immunol. Methods. 73:97.
11. Haba, S., and A. Nisonoff. 1985. Quantitation of IgE antibody by radioimmunoassay in the presence of high concentrations of non-IgE antibodies of the same specificity. J. Immunol. Methods. 85:99.
12. Haba, S., Z. Ovary, and A. Nisonoff. 1985 Clearance of IgE from serum of normal and hybridoma-bearing mice. J. Immunol. 134:3291.
13. Tung, A. S., S.-T. Ju, S. Sato, and A. Nisonoff. 1976. Production of large amounts of antibody in individual mice. J. Immunol. 116:676.
14. Mage, M. G., I. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with
and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. J. Immunol. Methods. 15:56.

15. Ishizaka, K. 1976. Cellular events in the IgE response. Adv. Immunol. 23:1.

16. Bennich, H. H., J. R. Ellerson, and T. Karlsson. 1978. Evaluation of basic serum IgE level and IgE antibody response in the rat by radioimmunoassay. Immunol. Rev. 41:261.

17. Bazin, H., and R. Pauwels. 1982. IgE and IgG2a isotypes in the rat. Prog. Allergy. 32:52.

18. Hamaoka, T., P. E. Newburger, D. H. Katz, and B. Benacerraf. 1974. Hapten-specific IgE antibody responses in mice. III. Establishment of parameters for generation of helper T cell function regulating the primary and secondary responses of IgE and IgG B lymphocytes. J. Immunol. 113:958.

19. Tada, T., K. Okumura, and M. Taniguchi. 1972. Regulation of homocytotropic antibody formation in the rat. VII. Carrier functions in the anti-hapten homocytotropic antibody response. J. Immunol. 108:1535.

20. Tada, T., and T. Takemori. 1974. Selective roles of thymus-derived lymphocytes in the antibody response. I. Differential suppressive effect of carrier-primed T cells on hapten-specific IgM and IgG antibody responses. J. Exp. Med. 140:239.

21. Takatsuki, K., K. Ishizaka, and T. P. King. 1975. Immunogenic properties of modified antigen E. III. Effect of repeated injection of modified antigen on immunocompetent cells specific for native antigen. J. Immunol. 115:1469.

22. Jardieu, P., T. Vede, and K. Ishizaka. 1984. IgE binding factors from mouse T lymphocytes. III. Role of antigen-specific suppressor T cells in the formation of IgE suppressive factor. J. Immunol. 133:3266.

23. Kishimoto, T., Y. Hirai, M. Suemura, and Y. Yamamura. 1976. Regulation of antibody response in different immunoglobulin classes. I. Selective suppression of anti-DNP IgE antibody response by preadministration of DNP-coupled mycobacterial. J. Immunol. 117:396.

24. Tung, A. S., N. Chiorazzi, and D. H. Katz. 1978. Regulation of IgE antibody production by serum molecules. I. Serum from complete Freund's adjuvant-immune donors suppresses irradiation-enhanced IgE production in low responder mouse strains. J. Immunol. 120:2050.

25. Katz, D. H., R. F. Bargatze, C. A. Bogowitz, and L. R. Katz. 1979. Regulation of IgE antibody production by serum molecules. IV. Complete Freund's adjuvant induces both enhancing and suppressive activities detectable in the serum of low and high responder mice. J. Immunol. 122:2184.

26. Hirashima, M., J. Yodoi, and K. Ishizaka. 1980. Regulatory role of IgE-binding factors from rat thymocytes. IV. Formation of IgE-binding factors in rats treated with complete Freund's adjuvant. J. Immunol. 125:2154.

27. Hirashima, M., J. Yodoi, and K. Ishizaka. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. III. IgE specific suppressive factor with IgE-binding activity. J. Immunol. 125:1442.

28. Suemura, M., J. Yodoi, M. Hirashima, and K. Ishizaka. 1980. Regulatory role of IgE binding factors from rat T lymphocytes. I. Mechanism of enhancement of IgE response by IgE-potentiating factor. J. Immunol. 125:1448.

29. Smith, W. G., and G. M. Butchko. 1986. Regulation of in vivo IgE biosynthesis in mice with complete Freund's adjuvant. Int. Arch. Allergy Appl. Immunol. 79:337.

30. Bergstrand, H., I. Andersson, R. Pauwels, and H. Bazin. 1985. Modulatory effects of Freund's adjuvant treatment on mast cell histamine release and homocytotropic antibody synthesis. Int. Arch. Allergy Appl. Immunol. 78:118.
31. Yefenof, E., V. M. Sanders, E. C. Snow, R. J. Noelle, K. G. Oliver, J. W. Uhr, and E. S. Vitetta. 1985. Preparation and analysis of antigen-specific memory B cells. *J. Immunol.* 135:3777.

32. Eisen, H. N., and G. N. Siskind. 1964. Variation in affinities of antibodies during the immune response. *Biochemistry.* 3:996.

33. Klinman, N. R. 1972. The mechanism of antigenic stimulation of primary and secondary clonal precursor cells. *J. Exp. Med.* 136:241.

34. Takemori, T., and K. Rajewsky. 1984. Specificity, duration and mechanism of idiotype suppression induced by neonatal injection of monoclonal anti-idiotype antibodies into mice. *Eur. J. Immunol.* 14:656.

35. Zubler, R. H., B. Benacerraf, and R. N. Germain. 1980. Feedback suppression of the immune response in vitro. II. IgVH-restricted antibody-dependent suppression. *J. Exp. Med.* 151:681.

36. Masuda, T., M. Miyama, K. Kuribayashi, J. Yodoi, A. Takabayashi, and S. Kyoizumi. 1978. Immunological properties of the receptors on lymphocytes. 5. Suppressive regulation of humoral immune responses by Fc receptor-bearing B lymphocytes. *Cell. Immunol.* 39:238.

37. Pisko, E. J., S. L. Foster, R. E. White, M. Panetti, and R. A. Turner. 1986. Suppression of a pokeweed mitogen-stimulated plaque-forming cell response by a human B lymphocyte-derived aggregated IgG-stimulated suppressor factor: suppressive B cell factor (SBF). *J. Immunol.* 136:2141.

38. Miyama, M., J. Yamada, and T. Masuda. 1979. Immunological properties of Fc receptors on lymphocytes. 6. Characterization of suppressive B cell factor (SBF) released from Fc receptor-bearing B cells. *Cell. Immunol.* 44:51.

39. Miyama-Iraba, M., T. Suzuki, Y.-H. Park, and T. Masuda. 1982. Feedback regulation of immune responses by immune complexes: possible involvement of a suppressive lymphokine by FcRy-bearing B cell. *J. Immunol.* 128:882.