

## T-CELL REGULATION OF MURINE IgA SYNTHESIS

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In studies of T-cell regulation of B-cell function, most workers have focused on immune responses involving the IgM, IgG, and IgE immunoglobulin classes and given little attention to responses involving the IgA immunoglobulin class. However, IgG and IgM responses on the one hand and IgA responses on the other hand may be independent of one another. For example, oral immunization of mice with ferritin (1) or sheep erythrocytes (2) produces an IgA response whereas the same antigens, given parenterally, result in an IgM and IgG response. One explanation for such divergence might be differences in the distribution or activity of regulatory T cells at mucosal as compared to systemic sites. Consistent with this idea, it has recently been shown that some protein antigens immunize when given parenterally but tolerize when given orally (3, 4) and the latter effect is mediated through T cells (5).

Little is known about the factors which control IgA responses aside from the fact that IgA responses are greatly reduced in the absence of T cells (6, 7). The present studies were initiated to determine the role of T cells in the control of IgA, IgM, and IgG synthesis in various mouse tissues. In the system employed, immunoglobulin synthesis *in vitro* was induced by a polyclonal activator, lipopolysaccharide (LPS),<sup>1</sup> and the resulting production and secretion of IgA, IgM, and IgG was then measured with specific double-antibody radioimmunoassays; in addition, the effects of T cells on immunoglobulin synthesis was determined by adding either concanavalin A (Con A) or Con A-pulsed T cells to LPS-driven indicator cultures. As a result of these studies, we have concluded that a separate set of T cells regulates IgA synthesis, and that IgA class-specific T cells are distributed unequally among various mouse tissues. These findings have important implications to the understanding of the IgA immune response.

### Materials and Methods

*Animals.* Balb/c mice were obtained from the National Institutes of Health animal facility and from the Frederick Cancer Research Center, Frederick, Maryland. Unless noted otherwise, they were used at 3–4 mo old.

*Cell Cultures.* Cells obtained from gentle teasing of various mouse tissues were washed four times through fetal calf serum to remove immunoglobulin. After one further wash in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 5% fetal calf serum, they were resuspended in complete media at  $1-2 \times 10^6$  cells/ml. Complete media contained RPMI 1640, 25 mM Hepes, 10% heat-inactivated fetal calf serum (Microbiologic Associates, Walkers-

<sup>1</sup> *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; Con A, concanavalin A; LPS, lipopoly saccharide; MLN, mesenteric lymph node; PLN, peripheral lymph node; PP, Peyer's patches; PWM-pokeweed mitogen.

ville, Md., lot No. 91495), 100 U/ml penicillin, 100  $\mu\text{g}/\text{ml}$  streptomycin, 50  $\mu\text{g}/\text{ml}$  gentamicin, 2 mM glutamine, and  $5 \times 10^{-5}$  M 2-mercaptoethanol. Triplicate cell cultures were placed in sterile 1-dram glass vials with loosely fitting plastic caps and incubated 7 days at 37°C in 5% CO<sub>2</sub> and humid air. The vials were then spun at 580 *g* for 10 min and the supernates carefully pipetted off. The supernates were stored at -20°C until radioimmunoassay.

**Mitogens.** The mitogens used and their suppliers were: Lipopolysaccharide S. typhimurium, lot 633443, Difco Laboratories, Detroit, Mich.; Con A lot 134, Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. The doses of LPS added are noted in the text. Con A was used at 4  $\mu\text{g}/\text{ml}$ , a dose which was found to be optimal for proliferation of spleen cells as measured by <sup>3</sup>H-thymidine uptake.

**Cell Separations.** T-cells were obtained using nylon column purification according to the method of Julius (8). Cells passing through the nylon were found to contain only 2–5% surface immunoglobulin-positive cells. Moreover, they proliferated well when stimulated with Con A but not when stimulated with LPS.

**Radioimmunoassays.** The double-antibody radioimmunoassay developed by Gleich was used (9).

**Purified Immunoglobulins.** IgA, IgM, and IgG were obtained from the following sources. IgA prepared from a TEPC 15 mouse myeloma, was the kind gift of Dr. Fred Mushinski of the National Cancer Institute. IgM was purified from a TEPC 183 mouse myeloma ascites fluid using Geon-Pevicon (B. F. Goodrich Cleveland, Ohio) block electrophoresis and Sephadex G-200 gel filtration (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N.J.). IgG was obtained from Miles Laboratories Inc., Miles Research Products, where it had been purified from mouse serum using chromatographic techniques.

**Antisera.** All antisera were obtained from rabbits immunized with the appropriate purified mouse immunoglobulins. Rabbit antimouse IgM, raised against a MOPC 104E myeloma IgM, was purchased from Litton Bionetics, Rockville, Md. (lot No. 2317517). Rabbit anti-mouse IgA was obtained by immunizing rabbits with purified MOPC 315 mouse myeloma IgA. Rabbit anti-mouse IgG was obtained from Miles Laboratories Inc., Miles Research Products, and was rendered monospecific by absorption on Sephadex-coupled MOPC 104E (IgM) and TEPC 15 (IgA) mouse myeloma proteins. Each antiserum was shown to be monospecific by Ouchterlony double-diffusion tests, by a lack of any binding of <sup>125</sup>I-labeled immunoglobulin of the other two classes, and by the absence of any inhibition of binding of the antiserum and its own class of <sup>125</sup>I-labeled immunoglobulin by an excess of unlabeled immunoglobulin of the other two classes.

**Radioiodination of Immunoglobulins.** <sup>125</sup>I was coupled to the purified immunoglobulins using a modification of the method of Hunter et al. (10).

**Assay Procedure.** Radioimmunoassays were done using an LKB 2071 (RIA) radioimmunoassay sample processor. In the assay procedure the following materials were added to 12 × 50 mm disposable polypropylene tubes: 0.5 ml of buffer (veronal buffered saline pH 7.2 with 10% fetal calf serum), 0.01–0.1 ml of sample or standard, 0.1 ml of diluted antiserum, 0.05 ml of a dilution of <sup>125</sup>I-labeled immunoglobulin (containing  $\approx 0.3$  ng). Each sample was done in duplicate, each standard in triplicate. After incubating this mixture at 10°C for 18–24 h, sheep anti-rabbit serum and normal rabbit serum were added to each tube at dilutions previously found to result in optimal precipitation. After an additional incubation for 24–48 h at 10°C, the tubes were centrifuged at 2,300 *g* for 30 min at 4°C and the supernates decanted. Radioactivity in the precipitate was counted in a Searle 1185 gamma scintillation counter (Searle Radiographics Inc., Des Plaines, Ill.). A standard curve was constructed for each assay by plotting the log of the percent specific counts bound against the log<sub>10</sub> nanograms of immunoglobulin standard added to the mixture. The curve was linear from 0.3 to 200 ng in the reaction mixture. From the percent of <sup>125</sup>I-immunoglobulin specific counts in the unknown samples, the concentration of Ig was read directly from the standard curve by an Olivetti P652 computer (Olivetti Corp. of America, N. Y.).

## Results

**Kinetics of Immunoglobulin Synthesis In Vitro.** When lymphoid cells are cultured in the presence of LPS, little or no IgM, IgG, or IgA is secreted into the culture supernate until the 3rd–4th d, at which time the rate of synthesis increases exponentially (Fig.

1). If the cells are irradiated on day zero there is little or no immunoglobulin present in the supernate on day 7. These results indicate that LPS is activating B cells to synthesize and secrete immunoglobulin and that the immunoglobulin measured is not simply a result of the shedding of cytophilic immunoglobulin or release of preformed immunoglobulin from plasma cells.

*LPS-Driven Immunoglobulin Synthesis in Various Tissues.* As shown in Fig. 2, IgM was synthesized by cells obtained from peripheral lymph node (PLN), spleen, mesenteric lymph node (MLN) and Peyer's patches (PP), with the greatest amount of synthesis always in cells obtained from spleen. In addition, IgG was synthesized to a roughly equal extent by cells obtained from all four tissues. Finally, IgA was synthesized by cells obtained from spleen, MLN, and PP cells but not by PLN cells; in this case, the ratio of IgA to IgM and the absolute amount of IgA synthesized was always highest in cells obtained from PP.

*Effect of Con A on Immunoglobulin Synthesis.* Con A has been found to be a useful probe of regulatory T-cell functions because it induces both T-cell suppression and T-cell help with the former predominating at mitogenic doses and the latter at submitogenic doses (11). In the present study, Con A was added at an optimal mitogenic dose to cultures of cells obtained from each of the four tissues mentioned above and the immunoglobulin synthesized was compared to that synthesized in the presence of LPS alone. As shown in Fig. 3 Con A induced a marked suppression of IgM synthesis in cultures of cells obtained from all four tissues. Similarly, Con A induced a net suppression of IgG synthesis in cultures of cells obtained from all four tissues, although to a somewhat lesser degree than that seen with IgM. In contrast, although Con A induced the suppression of IgA synthesis in the spleen cell cultures, this mitogen caused little change in the IgA synthesis observed in MLN cell cultures, and a large enhancement of the IgA synthesis observed in the PP-cell cultures. This enhancement of IgA synthesis in PP-cell cultures by the addition of Con A has been a consistent finding in many experiments and has ranged from a 2- to a 10-fold increase in IgA synthesis.

*Effect of Con A-Pulsed T Cells on Immunoglobulin Synthesis.* Con A is known to bind to B cells as well as T cells although it is mitogenic only for the latter. To ensure that the effects of Con A added to the culture media were not a result of a direct effect of Con A on B cells, purified spleen and PP T cells were pulsed for 40 h with Con A, washed thoroughly by centrifugation through fetal calf serum and then added to cultures of fresh cells obtained from the same tissue. The resultant cell mixtures then were incubated for 7 d in the presence of LPS and the effect of the pulsed T cells on immunoglobulin synthesis determined. It was found that Con A-pulsed spleen T cells added to fresh spleen cell cultures induced the suppression of IgM, IgG, and IgA synthesis (Fig. 4A), whereas Con A-pulsed PP T cells added to fresh PP-cell cultures induced the suppression of IgM and IgG, but the enhancement of IgA synthesis (Fig. 4B). These results agree with the earlier experiments in which Con A was simply added to the media, and confirm that the Con A effect is mediated through T cells. In addition, in data not shown, we have found that treatment of spleen cells by anti-T-cell antiserum plus complement abrogates the suppression seen when Con A is present in the media, further substantiating this point.

*Cell Responsible for the Enhancement of IgA Synthesis in PP Cultures.* The enhancement of IgA synthesis in PP cultures induced by Con A-activated PP T cells could be

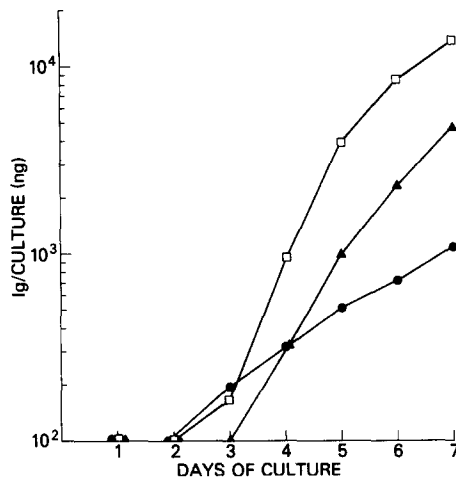


FIG. 1. Rate of appearance of immunoglobulin in supernates of cultured PP cells. Each point represents the mean of triplicate cultures, each culture containing  $10^6$  cells and LPS  $25 \mu\text{g/ml}$ . The standard deviation at each point was  $<15\%$  of the mean.  $\square$ , IgM;  $\blacktriangle$ , IgG;  $\bullet$ , IgA.

explained by an altered reactivity of the PP IgA B cell to T-cell signals: either a decreased sensitivity to T-cell suppression or an increased sensitivity to T-cell help. The alternate explanation is that the T-cell populations in spleen and PP differ in the ratio of T-cell helper to suppressor activity for IgA, with IgA T-cell helper activity predominating in the PP and IgA T-cell suppressor activity predominating in the spleen. To decide between these possibilities, spleen and PP T cells were pulsed with Con A as before, but then added to fresh indicator cell cultures of the opposite tissue. In these studies it was found that Con A-pulsed spleen T cells added to PP-indicator cell cultures suppressed IgM, IgG, and IgA synthesis (Fig. 5A); in contrast, Con A-pulsed PP T cells added to spleen indicator cell cultures suppressed IgM and IgG synthesis to an equal extent as compared to the spleen T cells but enhanced IgA synthesis (Fig. 5B). Thus, help or suppression of IgA synthesis occurred as a function of the T cell added and there was no evidence of an altered reactivity of the PP IgA B cell.

*Titration of Con A-Pulsed T Cells.* The previous experiments clearly establish that the PP T cells have a great deal of helper activity for IgA, much more than that present in other tissues. To eliminate the possibility that PP T cells might also have greater helper activity for IgM and IgG as well, this being masked for a greater sensitivity of IgM and IgG B cells to suppressor signals, Con A-pulsed PP and spleen T cells were directly compared over a range of T-cell:B-cell ratios. It was reasoned that under these conditions, particularly at low T-cell:B-cell ratios when suppressor T-cell effects are minimal, any difference between PP T cell and spleen T-cell helper activity for IgM and IgG would be evident. As can be seen in Fig. 6, there is no difference between Con A-pulsed spleen and PP T cells with regard to regulation of IgM and IgG, and thus, there is no evidence that PP T cells have more helper activity for these immunoglobulin classes. This contrasts with the regulatory activity of these two T-cell populations for IgA synthesis. Spleen T cells have helper activity for IgA synthesis at low T-cell:B-cell ratios but suppress IgA at high T-cell:B-cell ratios. PP T cells have greater IgA helper activity at every T-cell:B-cell ratio and do not suppress IgA

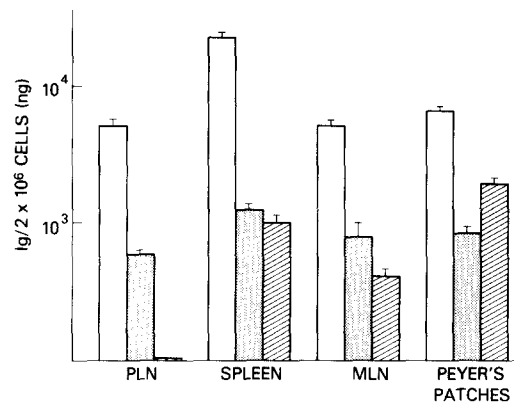


FIG. 2. Immunoglobulin synthesis in various mouse tissues. The bars represent the mean  $\pm$  SD of triplicate cultures. Each culture contained  $2 \times 10^6$  cells and LPS  $25 \mu\text{g/ml}$ . The tissue source is given beneath each set of bars. □, IgM; ▨, IgG; ▩, IgA.

synthesis at any T-cell:B-cell ratio. These results are not related to changes in cell density in the cultures because addition of nonpulsed T cells did not have these effects. From these studies we concluded that T-cell regulatory activity for IgA synthesis varies independently of that for IgM and IgG synthesis among different lymphoid tissues.

### Discussion

Because LPS is a potent B-cell mitogen, the amount of immunoglobulin produced by a given tissue under LPS stimulation can be used as a rough index of that tissue's total capacity to synthesize and secrete immunoglobulin. In this regard the variations seen among the various tissues in the synthesis of the different classes of immunoglobulin are of some interest. IgM and IgG were synthesized in all the tissues in roughly equivalent amounts with the exception that IgM synthesis was significantly higher in spleen cell cultures. IgA was synthesized by spleen, mesenteric lymph node, and PP, but the latter tissue usually produced the greatest amount and little or no IgA was made by peripheral lymph node. These results concerning IgA synthesis in various tissues are consistent with earlier work showing that PP cells, but not peripheral lymph node cells, were able to repopulate IgA plasma cells in irradiated allogeneic recipients (12).

It seemed possible that differences in regulatory T-cell activity was at least partially responsible for these differences in tissue immunoglobulin synthesis. In one set of experiments, Con A was added directly to the culture media at an optimal mitogenic dose. This resulted in a profound suppression of IgM in all four tissues, usually at least one log less synthesis as compared to cultures not containing Con A. Similarly, IgG was suppressed in all four tissues, although the degree of suppression was somewhat less, ranging from 50 to 90%. The effect on IgA synthesis was more variable: although there was suppression of IgA synthesis in spleen cell cultures, there was little change in the mesenteric lymph node cell cultures, and a marked enhancement of IgA synthesis in the PP cell cultures. In a second set of experiments, nylon-column purified T cells were pulsed for 40 h with Con A, washed thoroughly, and then added to fresh indicator cell cultures containing B cells and LPS. The results of these

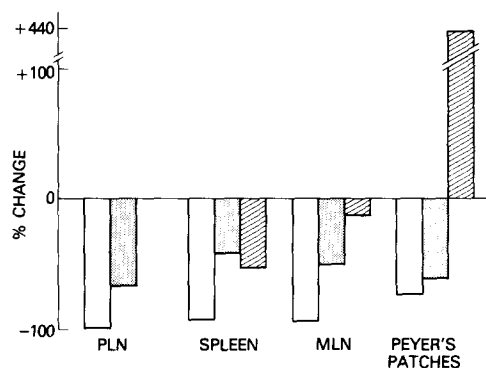


FIG. 3. Effect of Con A on LPS driven immunoglobulin biosynthesis in various mouse tissues. Con A was added to the culture media at  $4 \mu\text{g/ml}$ . The data are expressed as percent change from cultures containing LPS alone ( $25 \mu\text{g/ml}$ ). The tissue source is given beneath each set of bars.  $\square$ , IgM;  $\text{▨}$ , IgG;  $\text{▩}$ , IgA.

experiments were the same as those in which Con A was added directly to the media, i.e., Con A-pulsed spleen T cells suppressed IgM, IgG, and IgA synthesis, whereas Con A-pulsed PP T cells suppressed IgM and IgG synthesis but enhanced IgA synthesis. In sum, the data indicate that there are differences in T-cell regulatory activity for IgA but not for IgM or IgG in various murine tissues, with spleen containing regulatory T cells which predominately suppress IgA synthesis and PP containing regulatory T cells which predominately help IgA synthesis.

A possible objection to the above conclusion is that an altered reactivity of the PP IgA B cell was responsible for the enhancement seen after Con A activation of PP T cells: the enhancement could be explained on this basis if the PP IgG B cell was either hyposensitive to T-cell suppression or hypersensitive to T-cell help. In an effort to determine if this were the case, spleen and PP T cells, were pulsed with Con A as before, but then added to fresh indicator cell cultures of the opposite tissue; we reasoned that if there was an unusual reactivity of the Peyer's patch IgA B cell, the Con A-activated spleen T cell (which can suppress its own IgA B cell) should enhance the PP IgA B cell; similarly the Con A activated PP T cell should suppress the spleen IgA B cell. However, in these studies, the opposite result was found: the Con A-activated spleen T cell was able to suppress the synthesis of IgA by PP B cells just as it did the spleen IgA B cells and the PP Con A-activated T cells enhanced synthesis of IgA by the spleen B cells, just as it did the PP B cells. Thus, suppression or enhancement of IgA synthesis occurred as a function of the T cell added and was not a result of a difference in IgA B-cell sensitivity between spleen and PP. In addition, the mixed culture studies further established that PP T cells have a high ratio of helper to suppressor activity for IgA relative to that of the spleen T cells.

The independent variation of T-cell regulatory activities between spleen and PP for IgA on the one hand and IgM and IgG on the other seen in these experiments, strongly suggest that separate sets of T cells regulate these classes of immunoglobulins. However, from the data already discussed it remains possible that PP T cells have more helper activity for all immunoglobulin classes, but this was not seen in the case of IgM and IgG synthesis owing to more marked sensitivity of the IgM and IgG B cells to suppressor influences as compared to the IgA B cell. Therefore, a number of

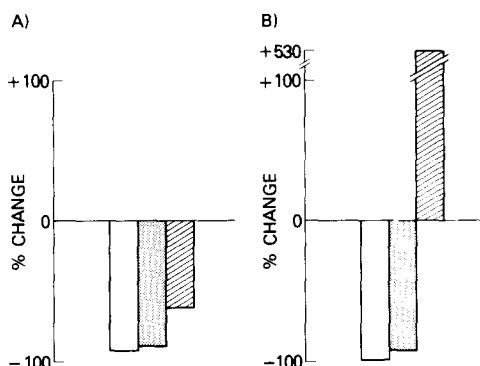


FIG. 4. Addition of Con A pulsed spleen or PP T cells to fresh indicator cell cultures of the same tissue. Nylon-purified T cells were pulsed with Con A  $8 \mu\text{g}/\text{ml}$  for 40 h, washed, and added to indicator cell cultures at a T-cell:B-cell ratio of 10:1. The data are expressed as percent change from indicator cell cultures not receiving Con A-pulsed T cells. LPS was present at  $10 \mu\text{g}/\text{ml}$ . A, Con A-pulsed spleen T cells added to fresh spleen cell cultures. B, Con A-pulsed PP T cells added to fresh PP cell cultures. □, IgM; ▨, IgG; ▩, IgA.

experiments were done in which varying numbers of Con-pulsed spleen or PP T cells were added to spleen indicator cell cultures, and the effect on IgM, IgG, and IgA synthesis determined for each T-cell dose. These experiments showed that the PP T cells had equivalent suppressor activity for IgM and IgG synthesis, but at the same time had markedly more helper activity for IgA synthesis than did spleen T cells. This result is difficult to reconcile with any model of immunoglobulin synthesis in which the same helper and suppressor T cells regulate IgM, IgG, and IgA with differences observed owing to variation in B-cell sensitivity. Instead, the data lead to the conclusion that there exist class-specific T cells regulating IgA as distinct from IgM and IgG synthesis.

Although this is the first demonstration of IgA-specific T-cell regulation under physiologic circumstances, IgA-specific suppressor T cells have been seen in pathologic states. Blaese et al. (13, 14), have shown that cells obtained from chickens rendered agammaglobulinemic by bursectomy and irradiation at hatching and injected into normal adult syngeneic birds will render the latter agammaglobulinemic. Moreover, Blaese et al., observed that as one decreased the number of cells transferred, various dysgammaglobulinemias were seen which were characterized by the absence of just one or two of the immunoglobulin classes. These dysgammaglobulinemias bred true on serial transfers to normal birds; that is, transfer of cells from a bird with IgA deficiency caused IgA deficiency in a second recipient and so on. Thus, it is clear that there are class-specific suppressor T cells present in this disease model.

IgA class-specific suppressor T cells have also been found in a subgroup of patients with selective IgA deficiency. Waldmann et al. (15), using a system of pokeweed mitogen (PWM) driven *in vitro* immunoglobulin synthesis, found that patients with selective IgA deficiency fell into two categories. The larger group did not secrete IgA into the culture with PWM stimulation, but had normal numbers of IgA-containing B cells; as a result these patients were felt to have a B-cell secretory defect. A second, small number of patients also did not secrete IgA into the culture medium with PWM stimulation and in addition, this group of patients did not have IgA-containing B cells. Coculture of these patients' cells with normal cells suppressed IgA but not IgM

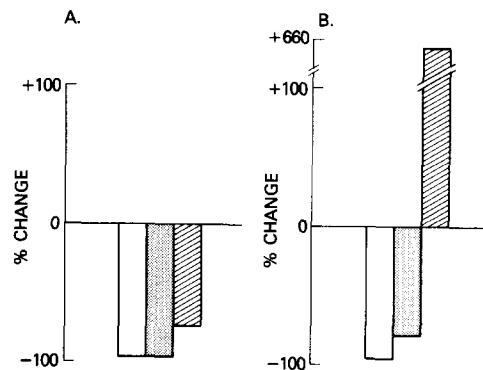


FIG. 5. Addition of Con A-pulsed spleen or PP T cells to fresh indicator cell cultures of the opposite tissue. Nylon purified T cells were pulsed with Con A  $8 \mu\text{g}/\text{ml}$  for 40 h, washed, and added to indicator cell cultures at a T-cell:B-cell ratio of 10:1. The data expressed as percent change from indicator cell cultures not receiving Con A-pulsed T cells. LPS was present at  $10 \mu\text{g}/\text{ml}$ . A, Con A-pulsed spleen T cells added to fresh PP indicator cell cultures. B, Con A-pulsed PP T cells added to fresh spleen indicator cultures. □, IgM; ▨, IgG; ▩, IgA.

or IgG synthesis by the normal cells. Thus, a subgroup of selective IgA-deficiency patients appears to be associated with IgA class-specific suppressor T cells. It is reasonable to assume that such class-specific regulatory T cells are also present and function in the same way in normals but are more difficult to demonstrate under physiologic circumstances.

One need not rely on studies of disease states for evidence of class-specific T-cell regulation, as there are many studies indicating that such class-specific T cells control normal IgE responses. Much of this data stems from the observation in the rat that an antigen given in alum or pertussis results in the production of both IgE and IgG antibodies, but the same antigen given in complete Freund's adjuvant (CFA) results in only IgG antibodies (16). This selective adjuvant effect appears to be mediated through T cells, because priming with a carrier in CFA can suppress a subsequent IgE (but not IgG) antibody response to a hapten on that carrier. In the rabbit this differential adjuvant effect on the IgE response can be shown to be mediated through the generation of helper T cells, i.e., carrier priming in CFA induces helper T cells which will cooperate with IgG B cells but not IgE B cells (17). In these studies, soluble IgE-specific T cell enhancing factors were found which were shown to be physically different from IgG specific T-cell enhancing factors (18). Finally in recent work in mice, Kishimoto, et al. (19) found that pre-administration of DNP-ovalbumin in alum-induced suppressor T cells which were both hapten and IgE class-specific. In summary, these experiments in the IgE system provide strong support for the concept of class-specific T-cell regulation of immunoglobulin synthesis.

The radioimmunoassay method used in the present study quantitates the isotype and not the idiotype of the antibody formed. However, one would predict from these data that if both isotype and idiotype were to be measured, the increased IgA-specific helper T-cell activity in the PP would magnify the IgA component of antibody formed whatever the idiotype. In this regard, the recent studies of Gearhart and Cebra (20) are of considerable interest. They used the *in vitro* splenic focus technique (21) to determine the frequency of clonal precursors of the anti-phosphorylcholine, anti-inulin, and anti-DNP idiotypes in PP and spleen. An increased proportion of IgA B-



## T-CELL REGULATION OF IgA SYNTHESIS

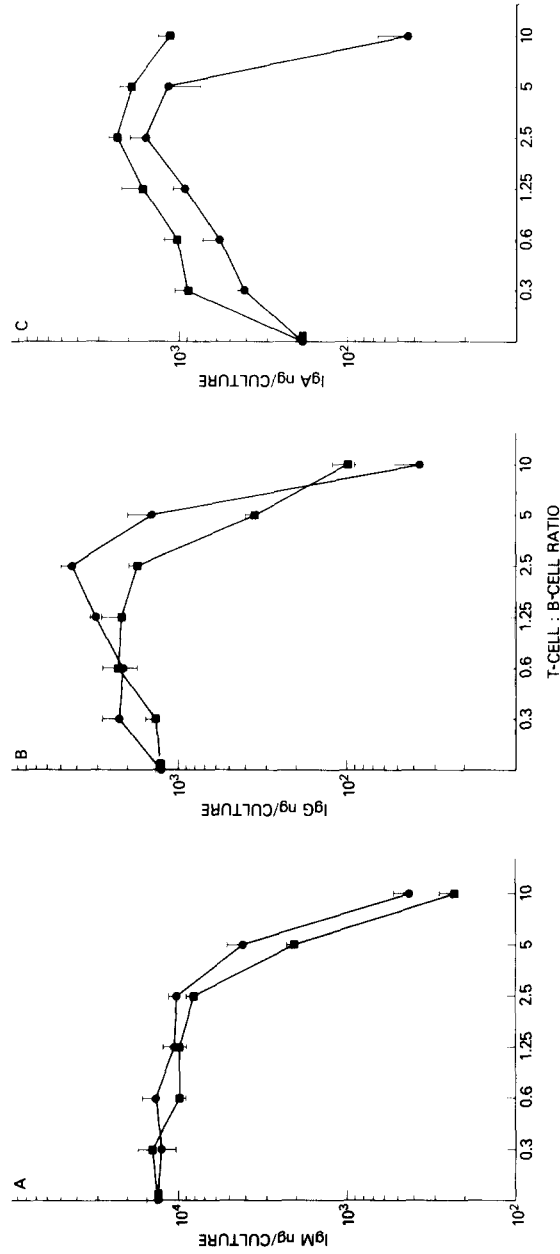


FIG. 6. Addition of graded numbers of Con A-pulsed spleen or PP T cells to fresh spleen cell-indicator cultures. Nylon-purified T cells were pulsed with Con A 8  $\mu$ g/ml for 40 h, washed, and added to indicator cell cultures at varying T-cell:B-cell ratios. All cultures contained 10<sup>6</sup> spleen cells plus LPS 10  $\mu$ g/ml. Each point represents the geometric mean of triplicate cultures and is expressed as nanograms of immunoglobulin per culture. The brackets represent one standard deviation. Spleen T cells, ●; PP T cells, ■. A, IgM data; B, IgG data; C, IgA data.

cell precursors for all three idiotypes was found in PP as compared to spleen. However, the absolute frequency of IgA-only B-cell precursors in the PP was increased only for anti-phosphorylcholine and anti-inulin, which are thought to be ubiquitous bacteria-associated antigens, but not for anti-DNP, a nonenvironmental antigen. In contrast, such an increase was not found in germ-free mouse PP, confirming the environmental dependence of the increase seen in PP of conventional animals. Finally, based on experiments in which PP precursors were able to make IgA in allogeneic recipients, Gearhart et al. concluded that PP contain an increased proportion of secondary or memory IgA B cells of anti-phosphorylcholine idotype. These results fulfill the prediction made above that the predominance of IgA-specific helper T-cell activity in the PP would magnify the IgA antibody response formed there. In addition, their findings in germ-free mice are consistent with observations made with the system reported here in that the predominance of IgA-specific helper T cells is dependent on environmental influence and such predominance is not seen in germ-free PP T cells (C. O. Elson, J. A. Heck, and W. Strober, unpublished data). A sequence of events can be postulated on the basis of the studies of Gearhart et al. and the studies reported here: luminal antigen, particularly bacterial antigen, preferentially activates PP T cells by an unknown mechanism to favor the generation of IgA-helper T cells. The latter then selectively magnify the IgA B-cell precursor pool and presumably also expand IgA-memory B cells.

The data reported here sheds new light on several earlier studies of the IgA-immune response. In the first place, it has been shown in previous work that PP do not contain plasma cells (22). However, it was shown in these studies that PP clearly have B cells which when stimulated *in vitro* are capable of synthesizing and secreting amounts of immunoglobulin comparable to that synthesized by other lymphoid tissues. An excess of suppressor T-cell activity cannot explain the lack of plasma cells in PP because, at least for IgA, helper T-cell activity is predominant in this tissue. A deficiency in PP macrophage function has been postulated (23), however, there is clearly enough macrophage function to generate antigen-specific helper T cells (24), a macrophage-dependent phenomenon (25). Because functional B cells, T cells, and macrophages are present in PP, the best explanation for the lack of plasma cells in PP appears to be that the primed B cells leave the patch after induction but before terminal differentiation (26).

Second, the present work is pertinent to the mechanisms underlying IgA-cell traffic. It has been shown that labeled mesenteric lymph node blast cells will be found in increased proportion in the intestine 24 h after an intravenous injection (27). Some have called this homing, a term which implies that there is a directed migration of IgA cells to the intestine via some special mechanism. There is, however, no evidence that homing in this sense really occurs, and the compensatory increase in IgM B cells in the intestine of IgA-deficient individuals raises doubt that it is specific for IgA if it does occur. A different mechanism can be postulated based on the following considerations: first, IgA is known to be highly T-cell dependent; second, IgA class-specific T cells exist; third, the ratio of helper to suppressor activities of these IgA-specific T cells varies among different tissues. From this, one can propose the following scheme: IgA B cells are induced by antigen in the PP, then leave there and drain into the mesenteric lymph node. They then randomly distribute throughout the body, but terminally differentiate and lodge only in sites where there is sufficient IgA T-cell

help. We have found that PLN cells, which synthesize little or no IgA in vitro also have little or no T-cell helper activity for IgA, consistent with this hypothesis (C. O. Elson and W. Strober, unpublished data). It remains to be shown that the intestinal lamina propria and other mucosal sites are rich in IgA T-cell helper activity.

Finally, these studies pertain to recent studies on antigen feeding in which it has been shown that, given the appropriate antigen and immunologic circumstances, PP can be a rich source of antigen-specific IgG-suppressor T cells (28). The predominance of helper T-cell activity for IgA in the PP as found here makes it likely that an antigen could induce a brisk IgA response at the same time it suppresses IgM or IgG. This makes sense for the body economy because it would at the same time decrease further absorption of antigen by secretion of IgA antibody and prevent untoward systemic reactions to the antigen by not allowing the formation of IgM and IgG antibody. We would predict that this phenomenon will be found when such antigen-feeding experiments are extended to include studies of the IgA response.

### Summary

In studies reported here, the polyclonal activator lipopolysaccharide was used to stimulate the synthesis and secretion of IgM, IgA, and IgG in cultures of mouse lymphoid cells. The total immunoglobulin of each class which resulted was measured by specific double-antibody radioimmunoassays. The effect of Con A-activated T cells from various tissues on such immunoglobulin synthesis was then assessed. Variations in regulatory T-cell activity among the various lymphoid tissues for IgA but not for IgM or IgG was observed. In particular, Peyer's patches T cells were found to contain a high level of IgA T-cell helper activity compared to that of spleen or peripheral lymph node. The independent variation of T-cell regulatory activity for IgA as compared to that for IgM and IgG among the different tissues is most consistent with there being a separate subset of T cells specifically regulating IgA. The significance of these findings for the understanding of the secretory immune system is discussed.

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### References

1. Crabbé, P. A., D. R. Nash, H. Bazin, H. Eyssen, and J. F. Heremans. 1969. Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunization with ferritin. *J. Exp. Med.* **130**:723.
2. Bazin, H., G. Levi, and G. Doria. 1970. Predominant contribution of IgA antibody-forming cells to an immune response detected in extra-intestinal lymphoid tissues of germ-free mice exposed to antigen by the oral route. *J. Immunol.* **105**:1049.
3. Phillip, G., and B. Boros. 1967. The induction of immunologic tolerance by antigen feeding. The effect of thymectomy on orally-induced tolerance. *J. Allergy.* **39**:167.
4. Thomas, H. C., and D. M. V. Parrott. 1974. The induction of tolerance to a soluble protein antigen by oral administration. *Immunology.* **27**:631.
5. Richman, L. K., J. M. Chiller, W. R. Brown, D. G. Hanson, and N. M. Vaz. 1978. Cellular mechanisms in immunologic tolerance induced by enterically administered soluble protein. *Gastroenterology.* **74**:1084.
6. Clough, J. D., L. H. Mims, and W. Strober. 1971. Deficient IgA antibody responses to arsanilic acid bovine serum albumin (BSA) in neonatally thymectomized rabbits. *J. Immunol.* **106**:1624.

7. Pritchard, H., J. Riddaway, and H. S. Micklem. 1973. Immune responses in congenitally thymus-less mice. II. Quantitative studies of serum immunoglobulins, the antibody response to sheep erythrocytes, and the effect of thymus allografting. *Clin. Exp. Immunol.* **13**:125.
8. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
9. Gleich, G. J., A. K. Averbach, and H. A. Swedlund. 1971. Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. Clin. Med.* **77**:690.
10. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of Iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)*. **194**:495.
11. Rich, R. K., and C. W. Pierce. 1973. Biological expressions of lymphocyte activation. I. Effects of phytomitogens in antibody synthesis *in vitro*. *J. Exp. Med.* **137**:205.
12. Craig, S. W., and J. J. Cebra. 1971. Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. *J. Exp. Med.* **134**:188.
13. Blaese, R. M., P. L. Weiden, I. Koski, and N. Dooley. 1974. Infectious agammaglobulinemia. Transmission of immunodeficiency with grafts of agammaglobulinemic cells. *J. Exp. Med.* **140**:1097.
14. Blaese, R. M., A. V. Muchmore, I. Koski, and N. J. Dooley. 1977. Infectious agammaglobulinemia: suppressor T-cells with specificity for individual immunoglobulin classes. In *Avian Immunology*. A. A. Benedict, editor. Plenum Publishing Co. N. Y. 155.
15. Waldmann, T. A., S. Broder, R. Krakauer, M. Durm, B. Meade, and C. Goldman. 1976. Defect in IgA secretion and in IgA specific suppressor cells in patients with selective IgA deficiency. *Trans. Ass. Amer. Physicians.* **89**:215.
16. Ishizaka, K. 1976. Cellular events in the IgE antibody response. *Adv. Immunol.* **23**:1.
17. Kishimoto, T., and K. Ishizaka. 1973. Regulation of antibody response *in vitro*. VI. Carrier-specific helper cells for IgG and IgE antibody response. *J. Immunol.* **111**:720.
18. Kishimoto, T., and K. Ishizaka. 1975. Immunologic and physicochemical properties of enhancing soluble factors for IgG and IgE antibody responses. *J. Immunol.* **114**:1177.
19. Kishimoto, T., Y. Hirai, M. Suemura, and Y. Yamamura. 1976. Regulation of antibody responses in different immunoglobulin classes. I. Selective suppression of anti-DNP IgE antibody response by preadministration of DNP-coupled mycobacterium. *J. Immunol.* **117**:396.
20. Gearhart, P., and J. J. Cebra. 1978. Differential expression of IgA by Peyer's patch B cells specific for environmental antigens. *Fed. Proc.* **37**:1396.
21. Klinman, N. R., and J. L. Press. 1975. The B cell specificity repertoire: Its relationship to definable subpopulations. *Transplant. Rev.* **24**:41.
22. Faulk, W. P., J. N. McCormick, J. R. Goodman, J. M. Yoffey, and H. H. Fudenberg. 1971. Peyer's patches: morphologic studies. *Cell. Immunol.* **1**:500.
23. Kagnoff, M. F., and S. Campbell. 1974. Functional characteristics of Peyer's patch lymphoid cells. I. Induction of humoral antibody and cell-mediated allograft reactions. *J. Exp. Med.* **139**:398.
24. Kagnoff, M. F. 1975. Functional characteristics of Peyer's patch cells. III. Carrier priming of T cells by antigen feeding. *J. Exp. Med.* **142**:1425.
25. Ishizaka, K., and T. Adachi. 1976. Generation of specific helper cells and suppressor cells *in vitro* for the IgE and IgG antibody responses. *J. Immunol.* **117**:40.
26. Kagnoff, M. F. 1977. Functional characteristics of Peyer's patch lymphoid cells. IV. Effect of antigen feeding on the frequency of antigen-specific B cells. *J. Immunol.* **118**:992.
27. McWilliams, M., J. M. Phillips-Quagliata, and M. E. Lamm. 1975. Characteristics of mesenteric lymph node cells homing to gut-associated lymphoid tissue in syngeneic mice. *J. Immunol.* **115**:54.
28. Mattingly, J. A. 1978. Antigen-specific suppressor T-cells formed in the Peyer's patches of rats after oral ingestion of antigen. *Fed. Proc.* **37**:1491.