MECHANISMS REGULATING IgA CLASS-SPECIFIC IMMUNOGLOBULIN PRODUCTION IN MURINE GUT-ASSOCIATED LYMPHOID TISSUES

I. T Cells Derived from Peyer’s Patches that Switch sIgM B Cells to sIgA B Cells In Vitro

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Substantial evidence is now available that Peyer’s patches (PP) are a major source of precursors of IgA-secreting plasma cells (1-3). These cells are generated in PP by exposure to antigen-specific or nonspecific stimuli. They then exit the PP, migrate through mesenteric lymph nodes, the thoracic duct, and systemic circulation, and finally localize in secretory tissues, where they differentiate into IgA-producing plasma cells (3). There is also considerable evidence that the IgA-predominant antibody production by PP B cells in gut-associated lymphoid tissues (GALT) is controlled by immunoregulatory T cells, such as class-specific helper and suppressor T cells (4-7). More recently, the existence of another type of regulatory T cell in PP, a contrasuppressor-inducer T cell, has been demonstrated, which suggests that IgA production is also influenced by a contrasuppressor regulatory circuit (8). Thus, immunoregulation of the mucosal immune response appears to be quite complicated.

As a new approach to the elucidation of the mechanisms governing IgA production in GALT we have studied the effect of cloned T cell lines on in vitro IgA synthesis. Accordingly, we have established concanavalin A (Con A)-induced cloned T cells from both murine PP and spleen and have determined the ability of these cells to regulate IgA immunoglobulin (Ig) synthesis and secretion by lipopolysaccharide (LPS)-driven PP B cells. In addition, we have analyzed the effect of co-cultured Con A-induced cloned T cells on the surface Ig (sIg) profile of PP and spleen B cells.

The data obtained strongly suggest that in murine PP there is a special type of T cell that causes switching of sIgM-bearing B cells directly to sIgA-bearing B cells. Such switch T cells do not operate on IgG-bearing B cells and do not cause proliferation and maturation of IgA-bearing B cells. Finally, the switch T cells cannot themselves cause terminal maturation of IgA-bearing B cells into IgA-producing plasma cells. The presence of such switch T cells in PP probably explains the high frequency of B cells committed to the exclusive generation of daughter cells expressing IgA, which is characteristic of B cells in the PP.

1 Abbreviations used in this paper: C, complement; C\text{H}2, heavy chain constant region of Ig; cIg, cytoplasmic immunoglobulin; Con A, concanavalin A; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GALT, gut-associated lymphoid tissues; IL-2, interleukin 2; LPS, lipopolysaccharide; 2-ME, 2-mercaptoethanol; PP, Peyer's patch(es); sIg, surface immunoglobulin; RIA, radioimmunoassay.
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Materials and Methods

Animals. Normal BALB/c female mice (8-16 wk old) and normal Sprague-Dawley female rats (8-10 mo old) were obtained from The Jackson Laboratory, Bar Harbor, ME, or from the animal production colonies of the National Institutes of Health, Bethesda, MD.

Mitogens. LPS, Salmonella typhimurium was purchased from Difco Laboratories, Detroit, MI, and Con A from Miles Laboratories Inc., Research Products Div., Elkhart, IN.

Production of Crude Interleukin 2 (IL-2). IL-2 used for T cell cloning and the routine maintenance of cloned T cell lines was made from the supernatants of Sprague-Dawley rat spleen cells stimulated by Con A (5 μg/ml) at 37°C for 36 h, according to a minor modification of the method reported by Gillis et al. (9).

Cell Suspensions. Single-cell suspensions were prepared from thymus and spleens (10). In some experiments adherent cells were removed from spleens by double plastic adherence. PP cells from murine small intestines were made with a new modification of a previously described technique (7) to obtain highly viable, noncontaminated lymphocyte suspensions. Briefly, a dissecting microscope was used to scrape away grossly removed PP from the serosa, and the minute follicular nodules thus obtained were further teased into single-cell suspensions in RPMI 1640 (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 5% heat-inactivated fetal calf serum (FCS) (Gibco Laboratories), 5 × 10^{-5} M 2-mercaptoethanol (2-ME) (Sigma Chemical Co., St. Louis, MO), and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, and gentamycin 50 μg/ml). The cell suspensions were then passed through a sterile 60-mesh stainless steel screen and an 18-gauge needle. Finally, the viable cells were separated on Ficoll-Hypaque gradients (Lympholyte M; Accurate Chemical & Scientific Corp., Westbury, NY). The viability of the cell suspensions thus obtained exceeded 95% (dye exclusion). They always contained >97.5% lymphocytes, <0.5% macrophages, and <2% mucosal surface epithelial cells as judged by Wright’s stain, nonspecific esterase stain (11), and ability to ingest latex beads (12), respectively. Morphologically identifiable plasma cells were not found in these cell populations.

Preparation of T and B Cells from Lymphocyte Suspensions. To obtain purified T cells and B cells, lymphocytes were first incubated in plastic petri dishes coated with goat anti-mouse Fab IgG (13); the cells not adherent to the plates were passed over nylon wool columns to obtain purified T cells (14), and the cells adherent to the plates were treated with a monoclonal rat anti-mouse Thy-1.2 (New England Nuclear, Boston, MA) and complement (C) (7) to obtain purified B cells. In some experiments the B cell populations were further purified with the use of an anti-mouse Lyt-1.2 alloserum (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) (15), as described below. Less than 0.5% of the T cells bore sIg as demonstrated by fluoresceinated rabbit anti-mouse polyclonal Ig and anti-mouse k plus l antiserum (N. L. Cappel Laboratories Inc., Cochranville, PA). Contamination by nonspecific esterase-positive cells was <0.1%. More than 99% of the B cells stained for sIg. Nonspecific esterase-stained cells in the latter cell population were <0.3%.

Preparation of Class-specific sIg-bearing B Cells from PP B and Spleen Cells by Positive and Negative Selection. Fractionation of PP B cells into class-specific sIg-bearing B cell subsets was carried out using plastic petri dishes coated with goat or rabbit chromatographically purified monospecific antibody (μ, γ, 2) (optimal concentration, 500 μg/ml) (N. L. Cappel Laboratories Inc.; and Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), as outlined in Fig. 1. The class specificity of these antibodies was verified by agar precipitin reactions. Each major class-specific sIg-bearing B cell-enriched fraction thus obtained was further depleted of two other major class-specific sIg-bearing B cells by treating appropriate monospecific antibodies and C, and then by spinning on a Ficoll-Hypaque gradient. Next, fractionation of spleen B cells into a sIgM-expressing B cell subset was carried out in a manner similar to that used for PP B cells. The subpopulations of class-specific sIg-expressing B cells thus obtained were at least 99.0% positive for a given isotype, as determined by direct immunofluorescence (see below).

T Cell Cloning. As shown in Fig. 2, before cloning, unseparated cells or T cell-enriched fractions (1 × 10^6/ml) from BALB/c PP and spleens were cultured with Con A (4 μg/ml) in complete culture medium in 75-cm² plastic culture flasks (25110; Corning Glass Works, Science Products Division, Corning, NY) at 37°C in an atmosphere of 5% CO₂ and 95% air for 4 d. After nearly 2 mo of in vitro maintenance, the Con A-induced blast cells were cloned by using
**Highly Purified PP B Cells**

- **Anti-IgM(μ)-Coated Petri Dish**
  - Adherent Cells
  - [Anti-IgG(γ) + Anti-IgA(α)] + C
    - Ficoll-Hypaque Gradient
  - slgM (= slgD)-Bearing PP B Cells

- **Anti-IgG(γ)-Coated Petri Dish**
  - Adherent Cells
  - [Anti-IgM(μ) + Anti-IgA(α)] - C
    - Ficoll-Hypaque Gradient
  - slgG (= slgD)-Bearing PP B Cells

- **Anti-IgA(α)-Coated Petri Dish**
  - Adherent Cells
  - [Anti-IgM(μ) + Anti-IgG(γ)] - C
    - Ficoll-Hypaque Gradient
  - slgA (= slgD)-Bearing PP B Cells

**FIG. 1.** Ig class-specific PP and spleen B cells were separated by using a "panning method" with class-specific anti-Ig-coated petri dishes (positive selection) and complement-mediated killing (negative selection).

- A limiting dilution technique (16). Briefly, cells were diluted into 96-well flat-bottomed microwells at estimated final concentrations of 3, 1, and 0.3 cells/well. Each well contained 0.2 ml of complete conditioned medium (supplemented with 20% IL-2 and 0.5 μg/ml additional Con A) containing the appropriate number of T cells as well as 2 × 10² irradiated (3,000 rad) syngeneic filler spleen cells. The cloned cell lines thus obtained were maintained in conditioned culture medium alone without the filler cells. For the present experiments a cloned line was selected from each tissue origin (K-14 from PP and K-17 from spleen). The clonal probabilities (17) of the K-14 and K-17 were 91 and 89%, respectively. All experiments using the primary cloned cells were performed ~4-8 mo after the initiation of the in vitro cell culture. In some experiments cloned T cells were irradiated at a dose of 1,500 rad.

**Antiserum Treatment of Cells.** BALB/c PP and spleen cloned cells, thymocytes, and virgin spleen cells (1 × 10⁶ cells/0.5 ml) were incubated with 1:200 dilution of monoclonal anti-Thy-1.2 (New England Nuclear), 1:20 anti-Lyt-1.2 (Accurate Chemical & Scientific Corp.), or 1:20 anti-Lyt-2.2 (Accurate Chemical & Scientific Corp.) for 45 min at 4°C. The cells were then treated with 1:8 low-tox rabbit C (Accurate Chemical & Scientific Corp.) for an additional 45 min at 37°C. All cell preparations were spun over Ficoll-Hypaque (Lympholyte M) to eliminate dead cells before killing. After treatment, a total of 400 live and dead cells were counted by means of trypan blue exclusion. Percentages of cells, killed and viable, were calculated according to the following formulae:

\[
\text{percent dead cells treated with antibody and C} = \frac{\text{percent dead cells treated with C alone}}{100} \times \frac{\text{percent specific killing}}{100} - \text{percent dead cells treated with C alone}
\]

\[
\text{percent viability} = 100% - \text{percent specific killing.}
\]

**Cell Cultures for Ig Biosynthesis.** Fractionated or unfractionated cells were suspended in RPMI 1640 supplemented with 10% FCS, 0.3% L-glutamine (Gibco Laboratories), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids (M. A. Bioproducts, Walkersville, MD), 5 × 10⁻⁵
Fig. 2. Method for long-term T cell cultures and cloning by limiting dilution, which uses irradiated syngeneic spleen filler cells and IL-2.

M 2-ME (Sigma Chemical Co.), 25 mM Hepes buffer, and antibiotics (complete culture medium). The cells were then cultured in small vials at 37°C in 5% CO<sub>2</sub>-95% air humid atmosphere for 7 d. Before culture the single-cell suspensions were washed four times through FCS to eliminate exogenous Ig. In some cases, cells received a dose of 1,500 rad from a cesium source (Gammaror M, Isomedix, Inc., Whippany, NJ) before culture.

Radioimmunoassays for Secreted Ig in Cell Cultures. The details of the ¹²⁵I-coupled double-antibody radioimmunoassay (RIA) used were described in the previous report of our laboratory (6). ¹²⁵I was coupled to each class-specific chromatographically purified Ig by using a modification of the method of Hunter et al. (18). Purified class-specific Ig used in the RIA were prepared as follows: IgA was prepared from TEPC 15 mouse myeloma ascites (Bionetics Laboratory Products, Litton Bionetics, Kensington, MD); IgM was prepared from HyGAL hybridoma cells, kindly provided by Dr. F. Mushinski, National Cancer Institute, National Institutes of Health; and IgG was prepared by ion exchange chromatography from mouse serum (N. L. Cappel Laboratories Inc.). Class-specific anti-mouse antibodies used in the RIA were as follows: rabbit anti-mouse IgA raised against purified MOPC 315 mouse myeloma IgA, rabbit anti-mouse IgM raised against purified MOPC 104 myeloma IgM (each obtained from Bionetics Laboratory Products, Litton Bionetics), and rabbit anti-mouse IgG raised against purified mouse monospecific IgG (N. L. Cappel Laboratories Inc.). Cross-reactivity of individual antisera with other classes of Ig was eliminated by appropriate absorption with Sephadex-coupled TEPC 15 (IgA), MOPC 104E (IgM), and monospecific mouse IgG. Class
specificity of each anti-mouse antibody was verified by the specificity of binding of 125I-labeled Ig.

Cell Cultures for Examination of Sequential Changes of Class-specific Ig and Clg Expression by PP or Spleen B Cells in the Presence or Absence of Cloned T Cells. Purified PP or spleen B cells (5 × 10⁶/2 ml per culture) were co-cultured with or without irradiated (1,500 rad) or nonirradiated cloned T cells (B/T ratio, 1:4) in the presence or absence of LPS (20 μg/ml). The cultures were kept in 5% CO₂ at 37°C for 3 d. At harvest the cultured cells were treated with anti-Thy-1.2 and C₃, and then layered on Ficoll-Hypaque and spun to eliminate dead cells.

Anti-Ig Antibodies. A fluorescein-isothiocyanate (FITC)-conjugated or unconjugated γ-globulin fraction of each rabbit or goat monospecific antibody directed against mouse major heavy chains (μ, γ, α) was obtained from N. L. Cappel Laboratories Inc., Kirkegaard & Perry Laboratories, and Bionetics Laboratory Products, Litton Bionetics. In indirect immunofluorescence, FITC-conjugated goat anti-rabbit polyvalent antiserum against mouse γ-globulins (N. L. Cappel Laboratories Inc.) was used. In some experiments, each chromatographically purified major heavy chain-specific Ig (N. L. Cappel Laboratories Inc. and Kirkegaard & Perry Laboratories) was also used for blocking studies. To control for nonspecific reaction with Fc receptors, FITC-conjugated F(ab')² fragments of anti-mouse IgM(μ), IgG(γ), and IgA(α) were also used in some experiments. The specificity of all the antibodies used was confirmed by Ouchterlony double diffusion studies.

Enumeration of Viable Fractionated and Unfractionated PP B Cells or Fractionated Spleen B Cells Co-cultured with or without Cloned T Cells. Before immunofluorescence studies, the number of viable cells in each culture was counted by trypan blue exclusion before culture and at harvest (5 d), as described above.

Immunofluorescence. Before immunofluorescence staining, the viable PP or spleen B cells obtained as above were treated with 0.05 M acetate buffer (pH 4.0) containing 0.085 M NaCl, 0.005 M KCl, and 0.03% human serum albumin (19), or were incubated at 37°C for 1 h, and then washed three times with RPMI 1640 to dissociate the cell-bound exogenous Ig present on the cells. For direct immunofluorescence, PP B cells were stained for Ig by incubating the cells (5 × 10⁶) with appropriate dilutions of FTTC-conjugated antibodies for 30 min on ice and then washing twice with cold RPMI 1640 containing 0.08% NaN₃. To stain cytoplasmic Ig (Clg), the cell suspensions (5 × 10⁶ cultured viable cells) were placed on a glass slide by using a Shandon cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, PA), air dried, and fixed with 95% ethyl alcohol plus 5% acetic acid. The spot of cells was then treated with the appropriate FITC-conjugated antibody, incubated for 30 min in a humidified chamber at room temperature, and finally mounted in Tris buffer-glycerol (pH 7.0). For indirect immunofluorescence, appropriate dilutions of each chromatographically purified heavy chain-specific goat or rabbit anti-mouse Ig preparation (see above) were used, and then chromatographically purified rabbit anti-goat or goat anti-rabbit polyclonal Ig conjugated to FITC was added. In each cell suspension (Slg) or smear (Clg) examined for immunofluorescence, 300–1,000 cells were viewed in both tungsten and ultraviolet light on a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, NJ) with a ×100 oil immersion objective and BG 38 and KP 490 excitation filters, coupled with a K 530 barrier filter.

Results

Properties of Con A-induced Cloned T Cells. Cloned T cells from PP (K-14) and cloned T cells from spleen (K-17) bore surface Thy-1.2 (≥99.5%) and Lyt-1 (≥99%) but not surface Lyt-2 (≤1%) antigens. In addition, study of these clones by direct immunofluorescence excluded the presence of membrane and cytoplasmic Ig (including κ and λ light chain determinants). Finally, the cells were negative for nonspecific esterase and could not mediate phagocytosis of latex beads.

Regulatory Function of Con A-induced Cloned T Cells in the Class-specific Ig Synthesis by PP B Cells

Ig production by LPS-driven PP B cells in the absence of cloned T cells. As shown in Fig. 3, secretion of IgM ranged from 7,350 to 10,370 ng/2 × 10⁶ PP B cells
per culture, and secretion of IgG ranged from 1,090 to 1,289 ng/2 × 10^5 PP B cells per culture. In contrast, little if any IgA was produced under these conditions (<47 ng/2 × 10^5 PP B cells per culture).

**Ig production by LPS-driven PP B cells in the presence of cloned T cells.** In IgM production, PP cloned T cells (K-14) markedly suppressed Ig synthesis by LPS-stimulated PP B cells proportionally to the number of T cells added (~88% inhibition at a 4:1 T/B cell ratio) (Fig. 3 A). Spleen cloned T cells (K-17) caused borderline suppression of IgM synthesis (~25% at a 4:1 T/B cell ratio, but no suppression at other T/B cell ratios) (Fig. 3 B). In IgG production, both PP- and spleen-derived T cell clones suppressed IgG synthesis and secretion in a dose-dependent fashion. At a 4:1 T/B ratio, both PP and spleen cloned T cells caused ~70% inhibition of IgG synthesis (Figs. 3 A and B). In IgA production, both PP and spleen cloned T cells enhanced IgA synthesis and secretion slightly (Figs. 3 A and B). The average amount of IgA produced was 285 ng/2 × 10^5 B cells at the highest PP cloned T/B cell ratio and 265 ng/2 × 10^5 B cells at the highest spleen cloned T/B cell ratio. This amount of IgA does not exceed the amount of IgM and IgG produced in the suppressed cultures (Figs. 3 A and B). Irradiation (1,500 rad) of cloned T cells did not change their capacity to affect Ig production (data not shown).

**Ig production by PP B cells in the absence of LPS, but in the presence of cloned T cells.** Both PP- and spleen-derived T cell clones enhanced B cell synthesis and secretion of all classes of Ig, even in the absence of LPS. However, this enhancement was quite minimal, ranging between 250 and 830 ng for IgM, between 135 and 370 ng for IgG, and between <26 and 199 ng for IgA, depending on the cloned T cell/B...
cell ratio (per $2 \times 10^5$ B cells). The effect on PP B cell Ig production of irradiated (1,500 rad) cloned T cells derived from PP and spleen was similar to that of nonirradiated cloned T cells (data not shown).

Distribution of PP B Cells Bearing Class-specific sIg and cIg Cultured in the Presence and Absence of LPS. The distribution of cells bearing the various surface Ig classes in unstimulated, precultured PP B cell populations (as obtained in the methods described above) is shown in Table I. The population was composed of ~80% IgM-bearing cells, up to ~20% IgA-bearing cells, and only a few percent sIgG-bearing cells. No cIg-bearing cells were detectable. When these PP B cells were cultured in vitro for 5 d, the absolute number and distributions of class-specific sIg- and cIg-bearing cells remained unchanged (Table II). In contrast, when cells were cultured for 5 d in the presence of LPS, the number of PP B cells increased approximately threefold, and the distribution of cells bearing the various class-specific sIg or cIg changed. sIgM-bearing cells decreased to ~29% and sIgG increased to ~17% of the cells (columns 1 and 2, Table II). Finally, an increase in the number of cells containing cytoplasmic IgM and IgG was noted, but no cells containing cIgA appeared. Thus, LPS stimulation of B cells results in the differentiation of IgM and IgG B cells into plasma cells secreting these Ig, but does not lead to differentiation of IgA cells.

Effect of Cloned T Cells on Expression of sIg and cIg in LPS-stimulated Cultures of Whole PP B Cell Populations. The addition of cloned T cells obtained from PP to cultures of whole PP B cell populations had a marked effect on sIg and cIg profiles, but only when such co-cultures also included LPS (Table II). Thus, in the absence of LPS the PP B cell populations co-cultured with cloned T cells obtained from PP (K-14) (4:1 T/B ratio) had much the same sIg and cIg percentages as B cells cultured alone, although there was some decrease in cells bearing sIgM and an increase in cells bearing sIgA in the co-cultured B cells (columns 1 and 3, Table II). In contrast, when LPS was present in the culture the addition of cloned T cells obtained from PP (K-14) had a dramatic effect on sIg and cIg profiles. There was a marked increase in the percentage of sIgA-bearing B cells and a corresponding drop in sIgM-bearing B cells. In addition, the percentage of cells containing cIgM and cIgG dropped. Finally,

| Table I |
| Distribution of Unprimed Murine PP B Cells Bearing Isotype-specific sIg and cIg before In Vitro Culture* |
| --- |
| **Percentage of total B cells bearing isotype-specific sIg** | **Percentage of total B cells bearing isotype-specific cIg** |
| **Mean** | **Range** | **Mean** | **Range** |
| sIgM | 76 | (69-80)‡ | cIgM | 0 | (0) |
| sIgG | 5 | (3-8) | cIgG | 0 | (0) |
| sIgA | 14 | (7-19) | cIgA | 0 | (0) |

* Highly purified B cell suspensions were prepared from pooled PP of 5-10 mice by anti-Fab adherence and subsequent treatment with anti-Thy-1.2 and C.
‡ Data from four separate experiments. At least 400 cells were counted at each given immunofluorescence examination. Borderline immunofluorescence-positive cells were eliminated from scoring.
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### Table II

Effect of PP Cloned T Cells (K-14) on Proliferation and Maturation of Isotype-specific PP B Cells in the Presence or Absence of LPS Stimulation In Vitro (5-d Culture)

| Population of PP B and plasma cells | Culture conditions of PP B cells* |
|------------------------------------|----------------------------------|
|                                    | PP B cells alone | PP B cells and LPS | PP B cells and cloned T cells | PP B cells, LPS, and cloned T cells |
| Total cell number (× 10^7/2 ml per culture) | 5.53 ± 0.28§ | 16.0 ± 0.57 | 4.66 ± 0.33 | 12.76 ± 0.39 |

| Isotype-specific slg- and clg-bearing cells | |
|-----------------------------------------------|-------------------|
| slgM                                          | 4.24 ± 0.22       |
| (76.7 ± 3.8%)†                                | (28.7 ± 2.2%)     |
| slgG                                          | 0.4 ± 0.15        |
| (7.3 ± 1.9%)‡                                | (16.7 ± 3.2%)     |
| slgA                                          | 0.75 ± 0.17       |
| (13.3 ± 2.6%)¶                               | (3.7 ± 1.0%)      |
| clgM                                          | 0                  |
| (0%)                                          | (25.3 ± 0.9%)     |
| clgG                                          | 2.46 ± 0.22       |
| (0%)                                          | (15.3 ± 0.9%)     |
| clgA                                          | 0                  |
| (0%)                                          | (0.6 ± 0.4%)      |

*5 × 10^7/2 ml per culture of highly purified PP B cells were initially used. In cases in which PP cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and C at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.

‡PP B cell/cloned T cell, 1:4.
§Expressed as (mean ± SEM) × 10^5 cells in three separate experiments.
¶Determined by immunofluorescence.
†Numbers in parentheses indicate percentage of total cells, expressed as mean ± SEM.

there was a small increase in the percentage of cells containing cIgA (columns 2 and 4, Table II).

The addition of spleen cloned T cells had a far different effect than the PP cloned T cells (Table III). In this case the percentage of cultured cells bearing slgM and slgA changed only marginally and that of cells bearing slgG increased to a significant extent compared with cells in cultures containing only LPS. Additionally, the percentages of cells containing clgM remained constant, and those of cells containing clgG dropped considerably. Finally, the percentage of cells containing IgA did not change.

In summary, it is clear that PP cloned T cells, but not spleen cloned T cells, markedly increased the appearance of slgA-bearing cells. Nevertheless, as in the studies of the secretion of Ig (mentioned above), cells containing IgA increased only marginally.

**Effect of Cloned T Cells on Expression of slg and clg in LPS-stimulated Cultures of Class-specific B Cell Populations Derived from PP**

To gain further insight into the effect of cloned T cells on expression of slg and clg, cells with defined isotypes were placed into culture with the PP or spleen cloned T cells.
Table III

Effect of Spleen Cloned T Cell (K-17) on Proliferation and Maturation of Isotype-specific PP B Cells in the Presence of LPS Stimulation In Vitro (5-d culture)

| Population of PP B and plasma cells | Culture conditions of PP B cells* |
|------------------------------------|----------------------------------|
|                                    | PP B cells and LPS               |
|                                    | PP B cells, LPS, and cloned T cells‡ |
| Total cell number ($\times 10^5/2$ ml per culture) | 15.13 ± 0.24§ | 13.64 ± 0.35 |
| Isotype-specific slg- and clg-bearing cells¶ | | |
| slgM | 4.40 ± 0.14 (29.2 ± 1.8%) | 4.29 ± 0.25 (31.4 ± 1.1%) |
| slgG | 2.38 ± 0.20 (15.7 ± 1.5%) | 3.12 ± 0.18 (22.8 ± 1.0%) |
| slgA | 0.50 ± 0.5 (3.3 ± 0.4%) | 0.90 ± 0.07 (6.6 ± 0.6%) |
| clgM | 4.57 ± 0.24 (29.5 ± 0.8%) | 4.07 ± 0.14 (29.9 ± 1.0%) |
| clgG | 2.53 ± 0.10 (16.8 ± 0.7%) | 0.52 ± 0.09 (3.8 ± 0.7%) |
| clgA | 0.02 ± 0.02 (0.15 ± 0.1%) | 0.01 ± 0.01 (0.09 ± 0.1%) |

* 5 × 10^5/2 ml per culture of highly purified PP B cells were initially used. In cases in which spleen cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and complement at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.
‡ PP B cell/cloned T cell, 1:4.
§ Expressed as (mean ± SEM) × 10^5 cells in three separate experiments.
¶ Determined by immunofluorescence.
Numbers in parentheses indicate percentage of total cells, expressed as mean ± SEM.

**Co-culture of slgM-bearing B cells with cloned T cells.** The effect of the addition of cloned PP T cells (K-14) to LPS-containing cultures of PP B cells bearing only IgM is shown in Table IV. The main effect is a dramatic increase in the percentage of cells bearing IgA, which occurs largely at the expense of cells bearing sIgG and cells that contain clgM and clgG. As in studies with whole unseparated B cell populations, cells containing clgA increase only slightly. In contrast, the addition of spleen cloned T cells (K-17) to similar B cell cultures does not result in a change in the number of sIgA-bearing cells or clgA-containing cells, although there is a modest drop in the number of cells containing clgG.

**Co-culture of slgG-bearing B cells with cloned T cells.** Adding PP-derived cloned T cells to LPS-containing cultures of PP B cells bearing only IgG causes little, if any, effect on the proportion of slg and clg cells as compared with cultures containing only LPS (Table V). Similarly, adding spleen cloned T cells (K-17) does not lead to marked changes, although in this case the percentage of sIgG cells increases, and the percentage of clgG cells decreases to a moderate degree. Thus, it is obvious that the cloned T cells derived from PP do not bring about changes in IgG B cells.

**Co-culture of sIgA-bearing B cells with cloned T cells.** The addition of cloned T cells obtained from either PP or spleen did not alter the number of sIgA
Table IV
Effect of PP (K-14) and Spleen (K-17) Cloned T Cells on Proliferation and Maturation of sIgM-bearing PP B Cells in the Presence of LPS Stimulation In Vitro (5-d Culture)

| Population of PP B and plasma cells | Culture conditions of sIgM PP B cells* | sIgM PP B cells and LPS | sIgM PP B cells, LPS, and PP cloned T cells‡ | sIgM B cells, LPS, and spleen cloned T cells‡ |
|-----------------------------------|----------------------------------------|-------------------------|---------------------------------------------|---------------------------------------------|
| Total cell number (× 10⁶/2 ml per culture) | 13.92 ± 0.59§ | 12.78 ± 0.30 | 12.87 ± 0.09 |
| Isotype-specific sIg-and cIg-bearing cells† | sIgM | 5.49 ± 0.27 | 4.59 ± 0.26 | 5.17 ± 0.28 |
| | | (40.3 ± 1.5%) | (35.9 ± 1.7%) | (40.4 ± 2.3%) |
| | sIgG | 2.24 ± 0.13 | 0.41 ± 0.09 | 3.32 ± 0.26 |
| | | (16.5 ± 1.4%) | (3.2 ± 0.7%) | (25.8 ± 1.7%) |
| | sIgA | 0.04 ± 0.02 | 5.48 ± 0.32 | 0.05 ± 0.03 |
| | | (0.3 ± 0.1%) | (42.9 ± 2.1%) | (0.4 ± 0.2%) |
| | cIgM | 3.92 ± 0.25 | 1.06 ± 0.13 | 3.37 ± 0.33 |
| | | (28.9 ± 1.9%) | (8.4 ± 1.0%) | (26.1 ± 2.4%) |
| | cIgG | 1.23 ± 0.17 | 0.21 ± 0.04 | 0.33 ± 0.08 |
| | | (9.0 ± 2.0%) | (1.7 ± 0.3%) | (2.6 ± 0.06%) |
| | cIgA | 0 ± 0 | 0.35 ± 0.12 | 0.009 ± 0.008 |
| | | (0%) | (2.7 ± 0.9%) | (0.07 ± 0.07%) |

* 5 × 10⁶/2 ml per culture of highly purified PP sIgM-bearing B cells were initially used. In cases in which PP or spleen cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and complement at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.
‡ sIgM-bearing PP B cell/cloned T cell, 1:4.
§ Expressed as (mean ± SEM) × 10⁵ cells in three separate experiments.
† Determined by immunofluorescence.
¶ Numbers in parentheses indicate percentage of total cells, expressed as mean ± SEM.

Cells in cultures (Table VI). In addition, no sIgM or sIgG cells appeared, nor did cells containing cIg of any isotype. These results clearly indicate that cloned T cells obtained from PP do not cause proliferation of sIgA B cells or terminal differentiation of such cells.

Effect of Cloned T Cells on Expression of sIg and cIg in LPS-stimulated Cultures of Class-specific sIgM-bearing B Cell Populations Derived from Spleen. Finally, to determine whether cloned T cells would induce a heavy chain switch in spleen B cells as well as in PP B cells, spleen B cells bearing sIgM were placed into co-culture with the PP or spleen cloned T cells. The effect of the addition of cloned PP T cells (K-14) or cloned spleen T cells (K-17) to LPS-containing cultures of the spleen B cells is summarized in Table VII.

The LPS-driven spleen B cells bearing sIgM, when cultured with cloned PP T cells, also expressed sIgA (but not cytoplasmic IgA) after 5 d of culture, as did the PP B cells bearing sIgM. However, the fraction of cells bearing sIgA, ~9%, was considerably lower than the corresponding fraction of B cells derived from PP subjected to the same treatment. Nevertheless, the fraction of cells bearing sIgG was not different, and that of cIgG was reduced to a slight extent from that observed in cells cultured with LPS alone.
TABLE V
Effect of PP (K-14) and Spleen (K-17) Cloned T Cells on Proliferation and Maturation of sIgG-bearing PP B Cells in the Presence of LPS Stimulation In Vitro (5-d Culture)

| Populations of PP B and plasma cells | Culture conditions of sIgG PP B cells* | sIgG PP B cells, LPS, and PP cloned T cells‡ (K-14) | sIgG B cells, LPS, and spleen cloned T cells‡ (K-17) |
|-------------------------------------|---------------------------------------|------------------------------------------------|--------------------------------------------------|
| Total cell number (× 10^5/2 ml per culture) | 5.24 ± 0.21§ | 4.95 ± 0.10 | 5.167 ± 0.088 |
| Isotype-specific sIg-and cIg-bearing cells† | | | |
| sIgM | 0 ± 0 | 0.004 ± 0.003 | 0.006 ± 0.006 |
| (0%)¶ | (0.08 ± 0.08%) | (0.1 ± 0.2%) |
| sIgG | 2.81 ± 0.16 | 2.60 ± 0.09 | 3.44 ± 0.03 |
| (53.6 ± 2.9%) | (52.6 ± 1.0%) | (66.6 ± 1.9%) |
| sIgA | 0.02 ± 0.02 | 0.008 ± 0.008 | 0.07 ± 0.02 |
| (0.3 ± 0.3%) | (0.17 ± 0.17%) | (1.4 ± 0.4%) |
| cIgM | 0 ± 0 | 0.03 ± 0.02 | 0 ± 0 |
| (0%)¶ | (0.6 ± 0.3%) | (0%) |
| cIgG | 2.21 ± 0.13 | 2.09 ± 0.13 | 1.42 ± 0.14 |
| (42.3 ± 2.0%) | (42.1 ± 2.1%) | (27.4 ± 2.3%) |
| cIgA | 0 ± 0 | 0.003 ± 0.003 | 0.008 ± 0.008 |
| (0%) | (0.07 ± 0.07%) | (0.16 ± 0.16%) |

*5 × 10^5/2 ml per culture of highly purified PP sIgM-bearing B cells were initially used. In cases in which PP or spleen cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and complement at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.

‡ sIgG-bearing PP B cell/cloned T cell, 1:4.
§ Expressed as (mean ± SEM) × 10^5 cells in three separate experiments.
¶ Determined by immunofluorescence.
† Numbers in parentheses indicate percentage of total cells, expressed as mean ± SEM.

The reverse result was obtained when sIgM-bearing spleen B cells were cultured with cloned spleen T cells. In this case no switch to IgA expression was observed, but there was a definite increase in the expression of sIgG (associated with a decrease in the expression of cIgG). In this case, however, the switch to sIgG expression was greater than that of PP B cells.

We conclude that the effects of PP cloned T cells and spleen cloned T cells on spleen B cells were quite parallel to the effects of these cloned T cells on PP B cells. However, PP B cells showed a greater tendency than spleen B cells to switching to IgA, and spleen B cells showed a greater tendency than PP B cells to switch to IgG. In both cases, however, this switch was dependent on a T cell switch signal from an appropriate T cell clone.

Discussion

In these studies we describe a Con A-induced cloned T cell line, derived from PP, which has a profound effect on LPS-induced Ig production by co-cultured PP B cells. Thus, compared with LPS-induced cultures not containing the cloned PP T cell line, the co-cultured B cells show (a) reduced production of IgM and IgG; (b) a decrease in the number of cells that express sIgM and sIgG; and, most importantly, (c) an increase...
in the number of cells that express sIgA. On the basis of these qualities, we postulate that the PP-derived T cells represent "switch T cells," which direct B cell differentiation away from IgM and IgG isotype expression and toward IgA isotype expression. Finally, the cloned PP T cell line is not dependent on the B cell source, since spleen B cells are affected by the cloned PP T cells in a qualitatively similar way.

Several facts concerning these putative switch T cells are noteworthy. First, the switch phenomenon was noted when the T cells were cultured with purified sIgM-bearing cells so that cells not bearing IgA are the apparent targets of the regulatory signals. The responding (switching) B cells appear to be partly organ specific, in that the combination of co-cultures of the PP B cells and PP cloned T cells had the most efficient yield of sIgA-bearing B cells. Second, purified sIgG-bearing PP B cells cocultured with the switch T cells did not switch to sIgA-bearing cells. This suggests that when cells, whatever their origin, are already committed to IgG isotype expression they are no longer capable of switching to IgA. In addition, this indicates that the switch T cells are not simply providing a nonspecific proliferative signal that moves DNA transcription to a more 3' location on the genome (20, 21). Third, the switch T cells did not lead to the proliferation of sIgA-bearing cells nor to IgA synthesis as assessed either by the appearance of significant amounts of IgA in culture supernatant or by the appearance of cells containing cIgA. Thus, the switch T cells do not act by merely causing proliferation of cells that have reached the level of IgA differentiation by random processes. Furthermore, these results indicate that the switch T cell is acting on B cells by affecting the course of DNA rearrangement rather than as a conventional helper cell that expands B cells already committed to a given isotype. The optimal switching process seems to be conducted by T and B cells from the same tissues.

### Table VI

**Effect of PP (K-14) and Spleen (K-17) Cloned T Cells on Proliferation and Maturation of sIgA-bearing PP B Cells in the Presence of LPS Stimulation In Vitro (5-d Culture)**

| Population of PP B and plasma cells | Culture conditions of sIgA PP B cells* | sIgA PP B cells, LPS, and PP cloned T cells (K-14)‡ | sIgA B cells, LPS, and spleen cloned T cells (K-17)‡ |
|-----------------------------------|--------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Total cell number (× 10⁶/2 ml per culture) | 4.12§ | 3.74 | 3.09 |
| Isotype-specific sIg- and cIg-bearing cells¶ | sIgM 0 0 0 | sIgA 4.08 (99.0%)¶ | sIgA 3.67 (98.0%) | sIgA 3.86 (99.2%) |
| sIgG 0 0 0 | cIgM 0 0 0 | cIgG 0 0 0 | cIgA 0 0 0 |
| sIgA 4.08 (99.0%)¶ | cIgA 0 0 0 | cIgA 0 0 0 | cIgA 0 0 0 |

*5 × 10⁶/2 ml per culture of highly purified PP sIgA-bearing B cells were initially used. In cases in which PP or spleen cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and complement at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.

‡ sIgA-bearing PP B cell/cloned T cell, 1:4.

§ Representative data are shown.

¶ Determined by immunofluorescence.

* Number in parentheses indicates percentage of total cells.
**Table VII**

Effect of PP (K-14) and Spleen (K-17) Cloned T Cells on Proliferation and Maturation of sIgM-bearing Spleen B Cells in the Presence of LPS Stimulation In Vitro (5-d Culture)

| Population of spleen B and plasma cells | Culture conditions of sIgM spleen B cells* | sIgM spleen B cells, LPS, and PP cloned T cells‡ | sIgM spleen B cells, LPS, and spleen cloned T cells‡ |
|-----------------------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                                         | Experiment 1§ | Experiment 2§ | Experiment 1 | Experiment 2 | Experiment 1 | Experiment 2 |
| Total cell number (× 10⁵/2 ml per culture) | 14.90 | 14.50 | 13.80 | 12.80 | 13.20 | 13.50 |
| sIgM bearing cells** | 5.62 | 5.73 | 5.09 | 4.28 | 3.80 | 4.02 |
| (37.7%)¶ | (39.5%) | (36.9%) | (33.4%) | (28.8%) | (29.8%) |
| sIgG | 3.25 | 3.21 | 2.98 | 2.66 | 5.35 | 4.82 |
| (21.8%) | (22.2%) | (21.6%) | (20.8%) | (40.5%) | (35.7%) |
| sIgA | 0 | 0 | 1.20 | 1.27 | 0 | 0 |
| (0%) | (0%) | (8.7%) | (9.9%) | (0%) | (0%) |
| cIgM | 3.49 | 4.09 | 2.28 | 2.73 | 3.10 | 3.86 |
| (23.4%) | (28.2%) | (16.5%) | (21.3%) | (23.5%) | (28.6%) |
| cIgG | 2.01 | 1.42 | 1.38 | 0.96 | 0.74 | 0.99 |
| (13.5%) | (9.8%) | (10.1%) | (7.5%) | (5.6%) | (4.4%) |
| cIgA | 0 | 0.01 | 0.06 | 0.06 | 0 | 0 |
| (0%) | (0.1%) | (0.8%) | (0.5%) | (0%) | (0%) |

*5 × 10⁵/2 ml per culture of highly purified spleen sIgM-bearing B cells were initially used. In cases in which PP or spleen cloned T cells were added, the mixed cultured cells were treated with anti-Thy-1.2 and complement at harvest to deplete the T cells. Viable cells of all cultures were obtained by centrifugation on gradients of Ficoll-Hypaque before each cell count and immunofluorescence study.

sIgM-bearing spleen B cells/cloned T cell, 1:4.

§ Each experiment was carried out in duplicate. The results are expressed as mean values.

¶ Determined by immunofluorescence.

That switch T cells result in sIgA-bearing but not IgA-producing plasma cells accords well with the well-known fact that no IgA-producing plasma cells are actually found in PP. In experiments not described here we have shown that stimulated T cells or factors derived from T cells can act on B cells preexposed to switch T cells, i.e., post-switch B cells, to cause sIgA-bearing cells to become IgA-producing cells. Thus, the likely sequence of events during in vivo differentiation of IgA cells is switch T cell-controlled B cell differentiation in PP followed by helper T cell-influenced terminal IgA B cell differentiation during traffic to mucosal sites or at mucosal sites. A small amount of IgA synthesis was in fact observed when either PP or spleen cloned T cells were cultured with B cells in the presence of LPS. This synthesis may result from cells in the PP B cell populations that have already undergone class switch and that are responding to nonspecific helper signals (T cell-replacing factor) secreted by residual T cells in the B cell populations or the cloned T cells themselves. Finally, the switch

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2 Kawanishi, H., L. Saltzman, and W. Strober. Mechanisms regulating terminal maturation of post-switch sIgA-bearing Peyer’s patch B cells in mice. Presented at conference on the Secretory Immune System (New York Academy of Sciences), held in New York, 4-7 May 1982.
HEAVY CHAIN SWITCH T CELLS

T cell governing IgA expression was found to be a property of two Con A-induced T cell clones derived from PP, but not from two other similarly produced T cell clones derived from spleen (all data not shown). It could therefore be assumed that IgA switch T cells are compartmentalized to the PP, thus accounting for the fact that the PP is a major source of IgA precursor cells.

The present data concerning the regulation of isotype switching by T cells should be viewed within the context of other recent studies in this area. Isakson and his co-workers (22) have found that supernatants obtained from cultures of Con A-induced alloreactive long-term T cell lines or of certain T cell hybridomas both augment IgG production in LPS-induced B cells. The factor acted on cells depleted of IgG-bearing cells and influenced the class of IgG produced (IgG1 selectively increased). These authors postulated that the cloned T cells produce a switch factor that influences B cell differentiation. In other studies, Mongini et al. (23) have shown that B cell responses to a T-independent antigen, trinitrophenyl-Ficoll, were influenced by the presence of T cells in a splenic focus assay. B cell clones produced more IgG2a in the presence of T cells than in their absence; furthermore, IgG2a-producing clones also produced IgM, and nearly all other IgG isotypes encoded 5' to the IgG2a gene. These authors concluded that T cells enhance the B cell switching process, but probably do so in a non-isotype-specific manner. The studies reported here are compatible with these data in that they also show that B cell switches can be influenced by T cells. However, in this case the influence of T cells on the switch to IgA does appear to be isotype-specific in that the cloned PP T cell acts only on IgM-bearing B cells, not on IgG-bearing B cells. Thus, it is possible that two general kinds of switch T cells occur, one that brings about IgM → IgG switches (which may or may not be IgG subclass specific) and one that brings about IgM → IgA switches. At the level of the genome the first type of switch T cell may promote sequential looping out of heavy chain constant region of Ig (C\(_{H}\)) segments corresponding to the various IgG subclass regions\(^2\) (20). The second type of switch T cell is qualitatively different in that it promotes bypassing of the entire IgG genomic region so that the C\(_{H}\)a becomes juxtaposed to the VDJ region (24, 25).

The cloned T cell line derived from spleen in these studies may in fact be a switch T cell of the type that can cause IgM → IgG switches. This cell caused preferential expansion of sIgG-bearing cells derived from both PP and spleen, whereas it reduced IgG but not IgM secretion by LPS-stimulated co-cultured B cells. These findings can be explained by either sIgM → sIgG switching or by sIgG → sIgG subclass switching, both occurring in the absence of terminal differentiation of IgG-bearing B cells. In either case, the switching process is unidirectional.

The data reported here are not in accord with the idea that IgA cells arise in PP simply because this tissue is the repository of cells having undergone multiple antigen-driven divisions accompanied by successive switches to progressively more 3' heavy chain DNA sites (26, 27). In this concept, such secondary B cells would more likely be IgA producers, because IgA is the most 3' heavy chain DNA segment. Instead, these data favor the concept that B cell differentiation into IgA expression does not involve sequential expression of each heavy chain class, but rather preferential and class-specific differentiation from IgM → IgA. In all, we conclude that PP are a source of IgA precursor cells because of the characteristic T cell environment present at this tissue site. In making this conclusion, we do not mean to imply that switch T cells are
absolutely necessary for IgA differentiation. In this regard, cells bearing and/or secreting IgA have been found in the bone marrow; i.e., at sites distant from PP and putative IgA-specific switch T cells. Thus, some IgA differentiation might occur in the absence of switch T cells, although this is not likely to be the predominant mode of mucosal B cell differentiation. We also do not mean to imply that B cells in PP may be different from those in other lymphoid organs in that they have a greater propensity to become IgA B cells when subjected to the appropriate differentiative influence (i.e., IgA-specific switch T cells). Indeed, we have seen that spleen B cell populations contain fewer IgA-expressing B cells after exposure to PP cloned T cells than do PP B cell populations. This may indicate that microenvironmental influence in PP other than switch T cells may be exerted directly on B cells to bring about IgA differentiation. However, this observation could also be explained by the possibility that slgM-bearing B cell populations derived from PP are already partially switched, and therefore give rise to more IgA-expressing cells than slgM-bearing B cells derived from spleen. The greater propensity of PP B cells to become IgA B cells rather than spleen B cells does not diminish the fact that both cell populations require a switch T cell derived from PP and that the latter is not capable of inducing IgG differentiation in other cell populations.

Much evidence has now accumulated that T cells can produce a variety of immunoregulatory factors or lymphokines (28-31). Thus, the mechanism of the class-specific heavy chain switch of B cells governed by switch T cells is assumed to be mediated via a switch factor in culture supernatants secreted by the latter effector cells. So far, however, we have not isolated the mediator from the supernatants studied.

Finally, as stated previously, the present findings do not define all aspects of IgA class-specific regulation in GALT. In fact, they suggest that the regulatory mechanisms governing IgA production involve at least two types of T cells: one, a PP switch T cell that exerts class-specific switching (μ to α), and the other, a helper T cell that operates on post-switch slgA-bearing B cells to bring about terminal differentiation. Such post-switch helper T cells have been identified in mesenteric lymph nodes and spleen and are the subject of a further report.

Summary

To explore mechanisms of T cell regulation governing mucosal IgA immune response, concanavalin A-induced cloned T cell lines from Peyer's patches (PP) as well as spleen were established. The cloned cell lines expressed Thy-1.2+, Lyt-1+2− and were radioresistant (1,500 rad). The capacity of the cloned T cells to regulate Ig synthesis was determined by measuring their effect on lipopolysaccharide (LPS)-induced polyclonal Ig synthesis by PP B cells. In initial studies Ig secreted by B cells was determined by double antibody radioimmunoassay. LPS in the absence of cloned T cells induced abundant amounts of IgM (average 8,860 ng/2 × 10^5 B cells) and IgG (average 1,190 ng/2 × 10^5 B cells), but little or no IgA. The addition of PP cloned T cells markedly suppressed production of IgM (88% at the highest T/B cell ratio, 4:1), but the addition of spleen cloned T cells suppressed only a little or not at all. IgG production was inhibited by both PP and spleen T clone cells (70% at the 4:1 T/B ratio), whereas IgA synthesis was enhanced by both clones, but only to a limited degree.
In subsequent studies the expression of class-specific surface Ig (sIg) and cytoplasmic Ig (cIg) on/in unseparated PP B cells as well as Ig class-specific PP B cells and spleen B cells during culture with or without the cloned T cells was determined by immunofluorescence. The major findings were as follows: (a) Compared with unseparated B cell cultures and cultures of purified slgM B cells derived from PP containing LPS alone, cultures containing LPS and PP cloned T cells showed a marked decrease in cIgM-, slgG-, and cIgG-expressing cells that was accompanied by a striking increase in slgA-bearing, but not cIgA-containing, cells. In contrast, unseparated B cell cultures and cultures of purified slgM B cells derived from PP containing LPS and spleen cloned T cells did not show any increase in slgA-bearing cells. (b) Compared with purified slgG-bearing PP B cell cultures containing LPS alone, purified slgG-bearing PP B cell cultures containing both LPS and PP cloned T cells showed no substantial change in slgG- or cIgG-expressing cells, and no slgA- or cIgA-expressing cells appeared. (c) Compared with slgA-bearing PP B cell cultures containing both LPS and PP cloned T cells showed no increased proliferation, and cIgA cells did not occur. Cultures of purified slgM B cells derived from spleen containing LPS and PP cloned T cells showed qualitatively similar changes. From these results we conclude that PP cloned T cells induced class-specific switching from slgM- to slgA-bearing B cells, whereas spleen cloned T cells lacked this property, although they may have induced an IgM → IgG or intersubclass IgG switch. These processes seem to be in part tissue dependent. Furthermore, the PP switch T cells appear to operate as true switch cells, which govern the pathway of DNA recombination events, rather than as classical helper cells, which act to expand already differentiated cells. Finally, these switch T cells probably account for the fact that PP are an important source of IgA B cells and also a major site of IgA heavy chain class switching during gut-associated mucosal B cell proliferation and differentiation.

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