TRANSFORMING GROWTH FACTOR β SPECIFICALLY
ENHANCES IgA PRODUCTION BY
LIPOPOLYSACCHARIDE-STIMULATED
MURINE B LYMPHOCYTES

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IgA is the major Ig isotype in mucosal secretions and constitutes the great majority of the Ig synthesized in mucosal tissues (1, 2). In contrast, IgA is a minor fraction of both the total serum Ig and the response by spleen and lymph node cells to most antigens (3). The physiological basis for this differential expression of IgA in various lymphoid tissues is not yet understood. A number of cytokines have been reported to preferentially enhance IgA production in vitro, including IL-5 (4-7), IL-2 (4), and IL-4 (8). In all cases, however, these factors cause a <10-fold increase in IgA relative to total Ig production, and none have yet been shown to be present at relatively high concentrations in mucosal tissues.

We have reported the cloning of a cDNA encoding murine IL-5 using as an assay the ability of IL-5 to enhance IgA production in cultures of LPS-stimulated B cells (7). The cloning strategy involved transient transfection of monkey COS7 cells with pools of cDNA clones from a T cell cDNA library, followed by assaying of the transfected cell supernatants for their IgA-enhancing activity. Since COS7 cells produced TGF- β , and TGF- β had been shown to inhibit human B cell responses (9), we examined the effects of TGF- β on the expression of individual Ig isotypes in cultures of LPS-stimulated B cells. These experiments led to the unexpected observation, described herein, that TGF- β substantially enhances IgA production, yet inhibits the production of most other Ig isotypes.

Materials and Methods

Mice. Female BALB/c ByJ mice were obtained from The Institute for Medical Research, San Jose, CA. Mice were used at 8-16 wk of age.

Cytokines. Porcine TGF- β 1 was obtained from R & D Systems, Minneapolis, MN. Murine IL-5 was purified from supernatants of the T cell clone MB2-1 by the method of Bond et al. (5). Murine rIL-2 was expressed in *Escherichia coli* and purified to >95% purity by Schering Research, Bloomfield, NJ. The specific activity of the IL-2 was 1.25×10^8 U/mg, where 1 U/ml supports one-half maximal growth of the T cell line HT2.

In Vitro Cultures and Assays. The basic in vitro culture system (10) and the purification of surface IgA⁺ (sIgA⁺) and sIgA⁻ Peyer's patch (PP) populations (11) have been described previously. Unless otherwise indicated, T cell-depleted populations were stimulated for 1 d with 8 μ g/ml *Salmonella typhosa* LPS at 10^6 cells/ml. The cells were then diluted twofold

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in medium containing the cytokine(s) to be tested and cultured for an additional 6 d in 0.2-ml round-bottomed microtiter plates. Supernatants were harvested at the end of the culture period and frozen until assayed. Each "culture" represents a pool of eight microtiter wells and all cultures were performed in duplicate or triplicate. The SD of the Ig levels in replicate cultures was <25% of the mean in most experiments. Isotype-specific ELISAs were performed on the culture supernatants with methods and reagents that have been described previously (10).

Results

TGF- β Enhances the Production of IgA. TGF- β has been reported to be a potent inhibitor of both proliferation and Ig production by *Staphylococcus aureus*-activated human B cells (9). A similar inhibitory effect by TGF- β on proliferation (Lebman, D. A., F. D. Lee, and R. L. Coffman, manuscript in preparation) and Ig production (Fig. 1) was observed in cultures of LPS-stimulated mouse B cells. However, analysis of the levels of individual Ig isotypes in cultures containing TGF- β gave an unexpected result. At concentrations of TGF- β that caused a $\sim$ 10-fold decrease in IgM, IgG1, IgG3, and total Ig production, the production of IgA was increased 10–20-fold (Fig. 1). Four different lots of porcine TGF- β 1 stimulated maximum IgA production at a concentration of 2 ng/ml, and this concentration was used in subsequent experiments.

IL-2 and IL-5, in Combination with TGF- β , Further Enhance IgA Production. Two other cytokines have been reported to enhance IgA levels in LPS-stimulated cultures in an isotype-specific manner, IL-2 (4) and IL-5 (4–7). We therefore tested combinations of these factors and TGF- β for their effects on the expression of IgA and other isotypes. The effects on six Ig isotypes of TGF- β , alone or in combination with IL-2 or IL-5, are shown in Table I. The results are typical of three such experiments. Supernatant IgA levels were increased as much as 40–50-fold in cultures containing TGF- β and IL-2, and IgA increased from $\sim$ 0.1% to 15–25% of total Ig produced (Table I). There appears to be synergy between the actions of these two factors, since the IgA level, induced by a combination of TGF- β and IL-2, was, in this example, nine times the sum of the IgA levels induced by the two factors separately. IL-5 also enhanced the TGF- β -induced IgA response, but to a lesser extent than IL-2. All cytokines were used at the optimum concentrations, as determined in dose titrations (data not shown). TGF- β caused substantial inhibition of the three major isotypes

FIGURE 1. Titration of TGF- β into cultures of LPS-stimulated, T cell-depleted spleen cells. Data for each isotype are expressed as the percent of the control response, i.e., the response obtained with no addition of TGF- β . This figure summarizes the results of one of three similar experiments.

TABLE I
Effect of TGF- β , IL-5, and IL-2 on Levels of Individual Ig Isotypes

LPS-stimulated T-depleted spleen cells +	Supernatant levels						IgA as percent of total Ig
	IgA	IgG2A	IgG2B	IgG1	IgG3	IgM	
	ng/ml						
Medium	85	40	381	1,220	4,532	63,900	0.12
TGF- β *	866	20	450	223	695	7,800	8.6
IL-5	380	45	814	2,000	4,870	115,000	0.13
TGF- β + IL-5	2,380	18	680	210	773	14,800	13
IL-2	303	290	990	3,200	4,000	103,000	0.27
TGF- β + IL-2	6,570	14	673	400	1,094	23,200	21

* TGF- β was used at 2 ng/ml, IL-5 at 1 ng/ml, and IL-2 at 10 ng/ml.

in these cultures, IgM, IgG1, and IgG3, and some inhibition of the small IgG2a response. IgG2b levels were relatively unaffected by TGF- β and, in some experiments, were enhanced two- to threefold. The addition of IL-2 or IL-5 partly reversed the suppression of total Ig production, in addition to further stimulating IgA production.

TGF- β Stimulates IgA Production from Surface IgA⁻ B Cells. Two principal mechanisms could explain the preferential enhancement of IgA relative to total Ig production by TGF- β . TGF- β could either cause an increase in the frequency with which B cells switch to IgA production, or preferentially stimulate the growth and/or differentiation of cells that switched to IgA independently of TGF- β . To distinguish which of these mechanisms might be responsible for the increase in IgA, PP B cells were separated by the FACS into sIgA⁻ and sIgA⁺ subpopulations, then cultured with

TABLE II
*TGF- β Enhances IgA Secretion by sIgA⁻ PP Cells
in LPS-stimulated Cultures*

B cell source	Additions	IgA ng/ml
Total PP	Medium	198
	TGF- β *	1,100
	IL-2	150
	TGF- β + IL-2	2,650
Total PP, stained, not sorted	Medium	54
	TGF- β + IL-2	790
sIgA ⁻ PP	Medium	21
	TGF- β	172
	IL-2	34
	TGF- β + IL-2	577
sIgA ⁺ PP	Medium	485
	TGF- β	67
	IL-2	500
	TGF- β + IL-2	216

Upon reanalysis, the sIgA⁻ population contained <0.5% sIgA⁺ cells and the sIgA⁺ population contained 6.6% sIgA⁻ cells.

* TGF- β was used at 2 ng/ml and IL-2 at 10 ng/ml.

LPS, TGF- β , and/or IL-2. Since 5–8% of the B cells in PP were sIgA⁺, the effects of TGF- β and IL-2 on both sIgA⁺ and sIgA⁻ subpopulations could be measured. Preliminary experiments had shown that PP and spleen cells respond similarly to TGF- β or TGF- β plus IL-2 (data not shown).

TGF- β enhanced IgA production by the sIgA⁻ population, and this was further augmented by the addition of IL-2 (Table II). This increase in IgA production does not appear to come from contaminating sIgA⁺ cells, since TGF- β inhibited IgA production by sIgA⁺ cells. However, the response of the sIgA⁺ population may not fully reflect their contribution to the response of unfractionated PP cells, since the treatment with anti-IgA antibodies consistently causes a two- to three-fold inhibition of the IgA response. A large increase in IgA production is likewise induced by TGF- β in splenic sIgA⁻ B cells and, in this case, staining of the unfractionated population with anti-IgA does not inhibit subsequent IgA production (Lebman, D. A., F. D. Lee, and R. L. Coffman, manuscript in preparation).

Discussion

The cytokine TGF- β proved to be a potent inhibitor of Ig production by LPS-stimulated murine B cells (Table I). Despite this, TGF- β induced a ~10–20-fold absolute enhancement of IgA production. The IgA response could be further enhanced by the addition of either IL-2 or IL-5, although IL-2 was more effective. Either cytokine caused a partial reversal of the inhibitory effects of TGF- β on most other isotypes. The net result was that cultures containing optimum concentrations of TGF- β and IL-2 produced 50–100 times as much IgA and only two- to threefold less total Ig than control cultures. Thus, IgA represented 15–25% of total Ig in cultures containing TGF- β and IL-2, compared with 0.1–0.2% of total Ig in cultures with no added cytokines.

A number of other cytokines and growth factors have been examined in LPS-stimulated B cell cultures, including IL-2 through IL-6, granulocyte-macrophage CSF, IFN- γ , TGF- α , epidermal growth factor, basic fibroblast growth factor, and insulin-like growth factor (4, and unpublished results). Only IL-5 and IL-2 cause significant specific stimulation of IgA, and these effects are small compared with those caused by TGF- β . TGF- β induces essentially the same responses in highly purified B cell populations and in the T cell-depleted spleen cell populations used in most of these experiments (data not shown), suggesting that both the IgA enhancement and the suppression of total Ig production reflect direct actions of TGF- β on B cells.

An important question about the mechanism of IgA enhancement by TGF- β is whether it affects the probability that B cells will switch to IgA or whether it specifically amplifies the response of B cells that have switched to IgA independently of TGF- β . This question was addressed by testing the response of sIgA⁻ and sIgA⁺ PP B cells to TGF- β or TGF- β + IL-2. Either addition caused a large increase in IgA production by sIgA⁻ B cells, suggesting that TGF- β enhances the switch to IgA production. Supporting evidence comes from experiments that demonstrated that TGF- β -enhanced IgA production comes from splenic B cells that are sIgA⁻ at the start of culture, but express readily detectable sIgA after 4 d of culture. Furthermore, TGF- β induced sterile mRNA transcripts from unrearranged C α loci before the appearance of detectable sIgA⁺ cells or secreted IgA (Lebman, D. A., F. D. Lee, and R. L. Coffman, manuscript in preparation).

TGF- β was originally described as a factor that caused anchorage-independent growth of fibroblasts (12). It is active on many cell types and can either stimulate or inhibit cellular proliferation and differentiation, depending on the cell type (reviewed in reference 13). It is produced by a wide variety of cell types (14), including platelets (12), activated B cells (9) and T cells (15), and macrophages (16). In general, TGF- β is quite inhibitory for lymphocytes, having been shown to inhibit proliferation and differentiation of B cells (9, 17) and T cells (15, 18, 19).

These experiments clearly show that polyclonally activated spleen and PP B cell populations, under certain conditions, can be induced to secrete IgA representing up to 25% of total Ig secretion. In principle, this type of response could account for the large number of sIgA⁺ B cells and the predominance of IgA secretion observed in gut-associated lymphoid tissues. The experiments reported here, however, provide no direct evidence that TGF- β actually plays a role in the development of IgA responses in mucosal tissue. Little information is available about TGF- β expression in gut-associated lymphoid tissues, although activated macrophages and lymphocytes secreting TGF- β should be present at the sites of ongoing mucosal immune responses.

Summary

The addition of TGF- β to cultures of LPS-stimulated murine B cells causes a ~10-fold enhancement of IgA production, yet causes a 10-fold decrease in total Ig production. IL-2 and, to a lesser extent, IL-5 synergize with TGF- β to further enhance IgA production and partially reverse the inhibition of total Ig production. IgA constitutes only ~0.1% of total Ig in LPS-stimulated cultures, but that percentage rises to 15–25% in cultures to which TGF- β and IL-2 are added. TGF- β induces a substantial increase in IgA production from sIgA⁺ B cells but inhibits IgA production by sIgA⁺ cells. This finding suggests that TGF- β acts as an isotype-specific switch factor for IgA.

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