Effects of Transforming Growth Factor β1 and Interleukin-2 on IgA Isotype Switching at the Clonal Level

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BACKGROUND

IgA is the predominant immunoglobulin isotype in mucosal secretions(1). It has been reported that a population of Peyer's patch T cells can selectively induce IgM bearing B cells to switch to surface IgA bearing B cells(2, 3). Further, IL-4, IL-5, and IL-6 alone and in combination, can significantly influence murine IgA B cell differentiation *in vitro*(4-7). However, it remains an open question which cytokines have a major role in class switching to the IgA isotype.

Recently, it has been reported that transforming growth factor β1(TGF β1) alone, or in combination with IL-2 increases IgA secretion by LPS-activated surface IgA negative(sIgA$^-$) murine spleen B cells while concurrently downregulating IgM and IgG secretion by such cells(8-11). In the present study, limiting dilution analysis was used to demonstrate, at the clonal level, that TGF β1 has siginificant activity as an IgA isotype switch factor.

CURRENT RESEARCH AND DISCUSSION

Murine spleen B cells, depleted of surface IgA bearing cells(i.e. sIgA-B cells) were prepared by using fluorescent activated cell sorter(Fig. 1). These highly purified sIgA-B cells were activated for 24 h by LPS(12.5 μg/ml) and then stimulated with optimal concentrations of either TGF β1 or recombinant IL-2(rIL-2) alone or with both TGF β1 and rIL-2. Seven days later, the number of IgA secreting cells was assessed using an Enzyme-Linked Immunospot(ELISPOT) assay and total IgA secretion was measured by Enzyme-Linked Immunosorbent assay(ELISA). As shown in Fig. 2, the number of IgA secreting cells in TGF β1 stimulated cultures was increased and there was a further increase in IgA secreting cells in TGF β1 and rIL-2 stimulated cultures(12). Increases in the numbers of IgA secreting cells were paralleled by increased total IgA secretion. In contrast, as shown in Fig. 3, the number of IgM and IgGl secreting cells was diminished in cultures stimulated with TGF β1 alone or with TGF β1 +rIL-2, and this was paralleled by a decrease in IgM and IgGl secretion. These findings suggest that TGF β1 alone or TGF β1 plus rIL-2 selectively increases the numbers of IgA secreting cells, resulting in the increase of total IgA production by LPS-stimulated sIgA-B cells.

Increased numbers of IgA secreting cells following stimulation with TGF β1± rIL-2, might reflect an increase in the frequency of switching of sIgA-B cells to IgA producing cells in culture. Alternatively, increased numbers of IgA secreting cells, after stimulation with those cytokines, might result from the increased proliferation of a few B cells in the population that were already committed to express the IgA isotype. To distinguish between those two possibilities, sIgA-spleen B cells were activated by LPS and then cultured at varing cell concentrations in mutiple replicate wells in a limiting dilution assay with TGF β1, rIL-2 or both TGF β1 and rIL-2. The ratioanale was as follows : If TGF β1 alone, or in combination with rIL-2, acted as an IgA switch factor, the fraction of replicate wells containing IgA secreting

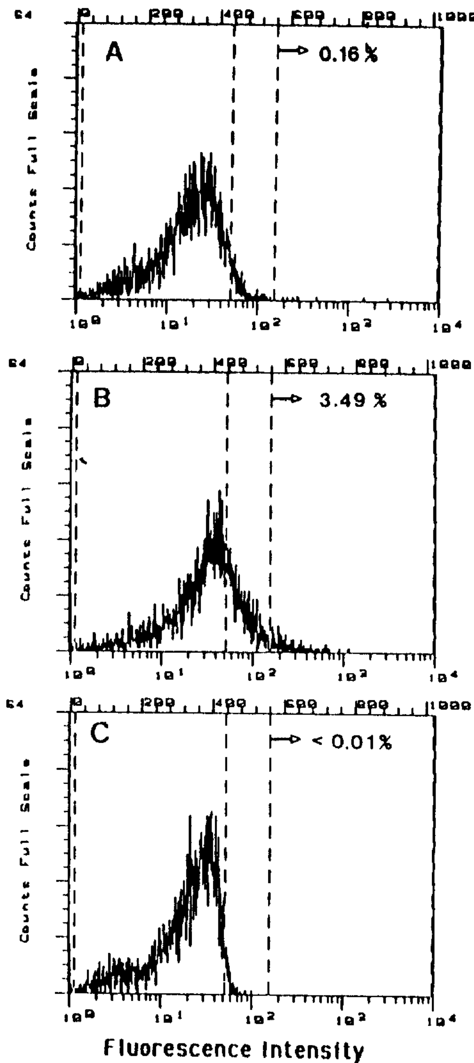

Fig. 1. Characterization of sIgA-spleen B cells prepared by flow cytometry. Murine spleen B cells were prepared as described before(8). The prepared B cells were incubated with goat anti-muse IgA as the primary antibody for 30 min at 4℃, washed and then labeled with FITC-conjugated rabbit F(ab')$_2$ anti-goat IgG as the sencondary antibody. Cells were sorted by a fluorescence activated cell sorter(FACSTAR, Becton Dickinson, Mountain View, CA) and the brightest 30%(first dot line) of the cells were excluded. B cell population, (A) Before sorting, the primary antibody control (normal goat IgG), IgA positivity(0.16%), (B) Before sorting, the primary antibody(goat anti-mouse IgA), IgA positivity(3.49%), (C) After sorting, the primary antibody(goat anti-mouse IgA), IgA positivity(<0.01%).

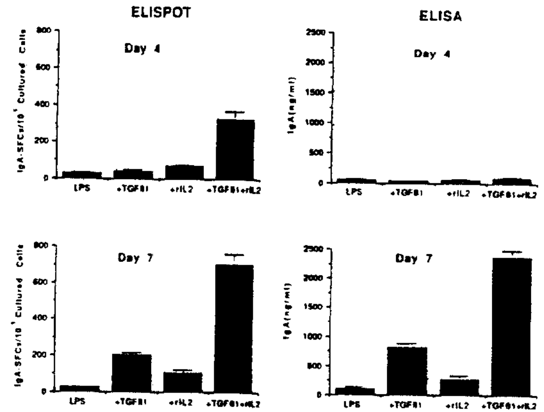

Fig. 2. Effect of TGF β1 and rIL-2 on numbers of IgA secreting cells and total IgA secretion. sIgA-spleen B cells were stimulated with LPS on day 0 of culture. Optimal concentrations of TGF β1 and/or rIL-2 were added on day 1. Supernatants and cells were harvested as indicated, on day 4 or 7 of culture. Numbers of IgA spot forming cells were assayed by ELISPOT(left) and total IgA secretion was assayed by ELISA(right). Data are means± SEM(vertical bars) of 6 cultures in 2 seperate experiments.

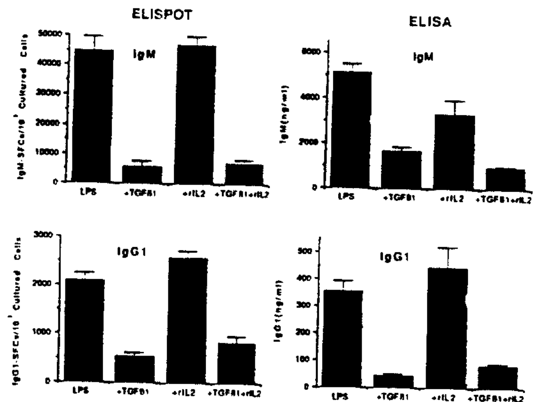

Fig. 3. Effect of TGF β1 and rIL-2 on numbers of IgM and IgGl secreting cells and the total IgM and IgGl secretion. Culture conditions were the same as described in Fig. 2. ELISA and ELISPOT assays were done on day 4. Data are means± SEM(vertical bars) of 6 cultures in 2 seperate experiments.

clones would be increased among cultures stimulated with those cytokines. Alternatively, if those cytokines increased the proliferation of B cells already committed to IgA production, the fraction

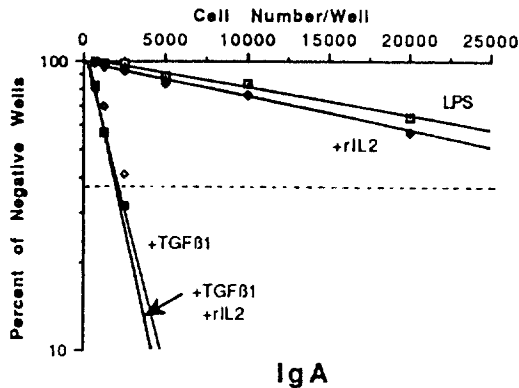

Fig. 4. Effect of TGF β1, rIL-2 and TGF β1 plus rIL-2 on the frequency of IgA secreting cels. For limiting dilution analysis, sigA⁻ spleen B cells were cultured in multiple wells at various cell densities. Cells were stimulated with LPS at the initiation of culture. On day 1, TGF β1, rIL-2 or TGF β1 plus rIL-2 were added to the LPS-stimulated cultures as indicated. Cells were harvested on day 6 and assayed by ELISPOt assay. Data are the means of 2 seperate experiments. Calculations to determine the frequency of B cells secreting IgA were based on Poisson distribution analysis as described before(13).

Table 1. Effects of TGF β1 and rIL-2 on the frequency of IgA secreting cells and the number of IgA secreting cells within clones[a].

Addition to Culture	IgA Secreting Cells	
	Frequency	Number of Cells/Clone
LPS	1/46,250	8± 1.9
+TGF β1	1/ 2,500	9± 0.6
+rIL-2	1/36,250	24± 1.4
TGFβ1+rIL-2	1/ 2,500	11± 1.4

[a]sIgA-spleen B cells were cultured in limiting dilution. Cells were stimulated with LPS alone or LPS and the indicated cytokines. LPS was added at the initiation of culture. Other additions were on day 1 of culture. Frequencies of IgA secreting cells are derived from Fig. 4 by interpolating to the cell number at which 37% of cultures are negative. This yields a cell number at which, on average, there is one IgA secreting cell per culture.

of wells with IgA secreting clones would be similar in control cultures and in cytokines stimulated cultures. However, the number of IgA secreting cells per clone predictably would be increased in the cytokine stimulated cultures.

As shown in Fig. 4, TGF β1 or TGF β1 and rIL-2 stimulated an approximately 20-fold increase in the frequency of IgA secreting B cell clones among populations of LPS-activated sIgA-B cells, without increasing IgA secreting B cells per clone. In contrast, stimulation with rIL-2 alone substantially increased the numbers of IgA secreting B cells per clone without any significant increase in the frequency of IgA secreting B cell clones.

In conclusion, TGF β1 has activity as an IgA switch factor. Thus, TGF β1 stimulated 20-fold increase in frequency of IgA secreting clones among populations of sIgA-spleen B cells but did not increase the numbers of IgA secreting cells within IgA producing clones. In contrast, as indicated by limiting dilution analysis, rIL-2 has little activity as an IgA switch. Rather, the major activity of IL-2 in the IgA response appears to be due to the stimulation of IgA secretion by B cells that become committed to the IgA isotype, or the stimulation of IgA B cell growth.

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