## *Brief Definitive Report*

# TRANSFORMING GROWTH FACTOR β INDUCES IgA PRODUCTION AND ACTS ADDITIVELY WITH INTERLEUKIN 5 FOR IgA PRODUCTION

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Maturation of activated B cells into Ig-secreting cells is induced by T cell-derived lymphokines that are able to induce B cell growth and/or differentiation (1). IL-5, formerly called T cell-replacing factor (TRF) or B cell growth factor II (BCGFII), has been shown to induce in vitro growth and differentiation of naturally activated B cells, as well as murine chronic B cell leukemia ($BCL_1$) cells (2–5), and to play a major role in T cell-dependent IgA production in the murine system (6–10). We and others (6, 8, 10) reported that IL-5 stimulates surface $IgA^+$ ($sIgA^+$) B cells, but not $sIgA^-$ B cells, giving rise to IgA-producing cells.

Transforming growth factor β (TGF-β) was originally described by its ability to confer anchorage-independent growth on nonmalignant fibroblasts, and is synthesized and secreted by a variety of cells (11). Known effects on the immune system include inhibition of lymphocyte proliferation, antibody production, and NK cell function (12, 13). Here we will report that TGF-β induces IgA production by LPS-stimulated murine B cells as well as suppression of IgM and IgG1 production.

## Materials and Methods

*Mice.* BALB/c mice were purchased from Japan SLC Inc., (Hamamatsu), and were maintained in the Laboratory Animal Facility of Kumamoto University.

*Cell Lines.* The chinese hamster ovary (CHO) cell line transfected with murine IL-5 cDNA was provided by Dr. T. Nishihara (Suntory Central Research Laboratories, Osaka) and was maintained in our laboratory.

*Antibodies.* TB13 rat IgG1 mAbs against mouse IL-5 were obtained as described (14). Rabbit anti-mouse IgA and anti-IgG1 antibodies were prepared as described (6). Purified anti-IgA and IgG1 antibodies were conjugated with horseradish peroxidase (HRPO) (CooperBiomedical, Inc., Malvern, PA) according to the described methods (15). Goat anti-mouse IgM and HRPO anti-mouse IgM antibodies were purchased from Zymed Laboratories (San Francisco, CA). Rabbit polyclonal anti-TGF-β IgG antibody was obtained from R & D Systems, Inc. (Minneapolis, MN). Affinity-purified goat anti-mouse IgA antibody was obtained from Southern Biotechnology Associates Inc. (Birmingham, AL).

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*Lymphokines.* Human recombinant TGF-β1 that had been previously described (16) was a kind gift of Genentech Inc. (South San Francisco, CA). IL-5 was purified from cultured supernatant of CHO cells as described (14). The specific activity of purified IL-5 was $2.2 \times 10^7$ U/mg protein.

*Antibody Production In Vitro.* T cell-depleted B cells were prepared as described (6). To deplete $sIgA^+$ B cells, T cell-depleted splenic B cells were incubated for 1 h at room temperature on bacteriological grade petri dishes (Clinical Test Ware, SH90-20; Iwaki Glass Co. Ltd., Tokyo) that had been precoated with purified goat anti-mouse IgA antibody (6). Cells nonadherent to the petri dishes were collected and used as $sIgA^-$ B cells. B cells thus obtained were suspended in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO) supplemented with $5 \times 10^{-5}$ M 2-ME, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% FCS (855; Flow Laboratories, Inc., McLean, VA), and were cultured in a 96-well microplate (25860; Corning Glass Works, Corning, NY) in the presence of 10 μg/ml LPS (Difco Laboratories Inc., Detroit, MI). Recombinant cytokines were added 24 h after the commencement of the culture, as described (6). The numbers of Ig-secreting cells were enumerated 5 d after the initiation of the culture by reversed plaque-forming cells (PFC) assay (6). For ELISA, cells were cultured for 7 d.

*ELISA Measurement of Ig Production.* ELISAs were done as described (15). Goat anti-mouse IgM antibody, rabbit anti-mouse IgG1 antibody, and goat anti-mouse IgA antibody were used for the solid phase antibody. HRPO anti-mouse IgA, HRPO anti-mouse IgM, or HRPO anti-mouse IgG1 antibody was used as the enzyme-linked antibody. *o*-Phenylenediamine and hydrogen peroxide were used as substrates. Dilutions of the mouse reference serum (Miles Scientific, Naperville, IL) were used to establish a standard curve.

## Results

*TGF-β Induces IgA Production of LPS-stimulated B Cells.* T cell-depleted B cells were stimulated with LPS for 5 d, and TGF-β, IL-5, or TGF-β plus IL-5 was added to the culture on day 1. As shown in Fig. 1, addition of TGF-β or IL-5 enhanced IgA PFC responses. IgA responses reached a maximal level when 1 ng/ml of TGF-β was added, and were higher than those induced by IL-5. In this case, cell recoveries after the culture with TGF-β were decreased to ~50% of control cultures (data not shown). Addition of both TGF-β and IL-5 caused more striking IgA production than that induced by either of cytokines. No significant IgA production was observed in the absence of LPS.

To evaluate whether TGF-β selectively enhances IgA production, the effect of TGF-β on IgM and IgG1 production was also examined. As shown in Table I, addition of IL-5 enhanced IgA, IgM, and IgG1 production by LPS-stimulated B cells. TGF-β augmented IgA production and acted additively with IL-5, whereas it showed a

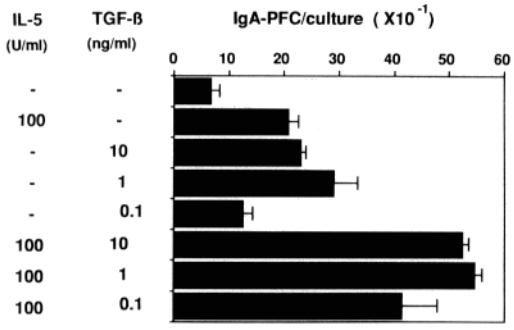


FIGURE 1. Enhancement of polyclonal IgA production of LPS-stimulated B cells by TGF-β. T cell-depleted B cells ($10^5$/well) were stimulated with LPS (10 μg/ml) for 5 d. Either IL-5 (100 U/ml), TGF-β (1 ng/ml), or IL-5 plus TGF-β was added on day 1. After the culture the numbers of IgA-producing cells were enumerated by the reversed PFC assay. Results were expressed geometric means of IgA PFC of triplicate cultures and SDs.

TABLE I

*TGF-β Enhances IgA Production by LPS-stimulated B Cells*

| TGF-β | IgA | | IgM | | IgG1 | |
|---|---|---|---|---|---|---|
| | (−) IL-5 | (+) IL-5 | (−) IL-5 | (+) IL-5 | (−) IL-5 | (+) IL-5 |
| | | | *ng/ml* | | | |
| 0 | 84 | 440 | 25,000 | 56,000 | 640 | 740 |
| 100 | 290 | 752 | ND | ND | ND | ND |
| 10 | 440 | 926 | 5,160 | 12,400 | 48 | 52 |
| 1 | 448 | 1,580 | 11,800 | 24,400 | 120 | 76 |
| 0.1 | 170 | 1,180 | 18,400 | 36,400 | 360 | 270 |
| 0.01 | 92 | 320 | ND | ND | ND | ND |

T cell–depleted B cells ($10^5$/well) were cultured with LPS (10 μg/ml) for 7 d. Either TGF-β, IL-5 (100 U/ml), or TGF-β plus IL-5 was added on day 1. After the culture IgA levels in supernatants were titrated by ELISA. Results were expressed as mean value of triplicate cultures.

significant inhibitory effect on IgM and IgG1 secretion irrelevant to the existence of IL-5. The augmenting effect of IgA production by TGF-β was abrogated by anti-TGF-β antibody (Table II), whereas IL-5-induced IgA synthesis was not affected. These results indicate that the effect we are looking at is caused by TGF-β, and TGF-β is not involved in IL-5-mediated IgA production.

TABLE II

*Effect of Anti-TGF-β Antibody on IgA Production*

| Stimulant | | | IgA antibody production |
|---|---|---|---|
| TGF-β | IL-5 | Anti-TGF-β | |
| *ng/ml* | *U/ml* | *μg/ml* | *ng/ml* |
| 0 | 0 | 0 | 84 |
| 1 | 0 | 0 | 480 |
| 1 | 0 | 25 | 88 |
| 0 | 100 | 0 | 286 |
| 0 | 100 | 25 | 265 |

Anti-TGF-β antibody or control antibody was added to the culture when TGF-β or IL-5 was added on day 1. Results were expressed mean value of triplicate cultures.

We analyzed the time course of TGF-β and IL-5 for their additive effects on IgA production. TGF-β was added on day 2 or 3 to the culture in the presence of IL-5 (Fig. 2, groups 7 and 8). In other groups, IL-5 was added to the culture on day 2 or 3 (groups 5 and 6) in the presence of TGF-β. Either TGF-β, IL-5, or TGF-β plus IL-5 was added to the culture on day 1 (groups 2–4). Simultaneous addition of TGF-β and IL-5 induced maximal IgA production (group 4). Addition of IL-5 on day 2 or 3 induced a maximum IgA production in the presence of TGF-β (groups 5 and 6). In contrast, addition of TGF-β on day 2 or 3 showed no additive effect by antecedent addition of IL-5 (groups 7 and 8).

*TGF-β Acts on sIgA⁻ B Cells to Induce IgA Production.* sIgA⁻ B cells were separated

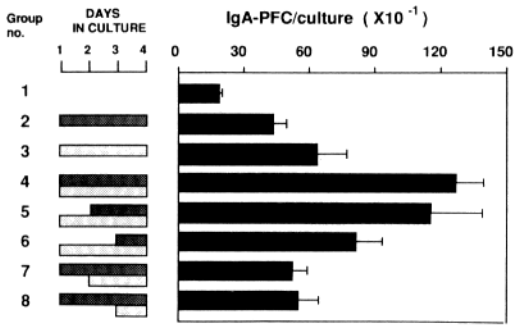


FIGURE 2. Kinetic analysis of synergy between TGF-β and IL-5. T cell-depleted splenic B cells were cultured with LPS as described in Fig. 1. TGF-β (1 ng/ml) or IL-5 (100 U/ml) was added to the culture as indicated. (▦) Time period during which TGF-β was present; (■) period during which IL-5 was present.

from sIgA$^+$ B cells as described in Materials and Methods. Contamination of sIgA$^+$ B cells in the sIgA$^-$ B cell population was <0.5%. Because sIgA$^+$ B cells thus obtained were not enough to carry out further analysis, either unfractionated or sIgA$^-$ B cells were cultured with LPS and cytokines to be tested. IgA production was observed even when sIgA$^-$ B cells were cultured with TGF-β (Table III) and its level was comparable with that observed in the culture of unfractionated B cells with TGF-β. However, sIgA$^-$ B cells did not differentiate into IgA-producing cells in response to IL-5. IL-5 showed an additive effect with TGF-β on IgA production by sIgA$^-$ B cells, whereas both IgM and IgG1 production induced by IL-5 were inhibited by TGF-β, and there were few differences between the unfractionated and sIgA$^-$ B cells regarding their production (data not shown).

TABLE III

*sIgA$^-$ B Cells Responded to TGF-β in the Presence of LPS*

| Stimulant | | IgA produced by B cells | | | |
|---|---|---|---|---|---|
| | | Unfractionated | | sIgA$^-$ | |
| TGF-β | IL-5 | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| *ng/ml* | *U/ml* | *ng/ml* | | | |
| 0 | 0 | 64 | 46 | <20 | <20 |
| 10 | 0 | ND | 220 | ND | 210 |
| 1 | 0 | 246 | 184 | 690 | 220 |
| 0 | 100 | 160 | 110 | 52 | <20 |
| 1 | 100 | 890 | 435 | 1,320 | 560 |

Either unfractionated or sIgA$^-$ B cells ($10^5$/well) were stimulated with LPS (10 μg/ml) for 7 d. Either TGF-β, IL-5, or TGF-β plus IL-5 was added on day 1 of the culture. After the culture IgA levels in cultured supernatants were determined by ELISA. Results were expressed as mean of triplicate cultures.

## Discussion

This paper documents the effects of TGF-β on IgA production. TGF-β selectively enhanced polyclonal IgA formation of LPS-stimulated B cells and acted additively with IL-5 for IgA production only when TGF-β was added to the culture simultaneously with IL-5 or before IL-5 (Figs. 1 and 2, and Tables I and II). More interestingly, sIgA$^-$ B cells responded to TGF-β, resulting in IgA-secreting cells (Table III),

whereas IL-5 was ineffective. Several investigators, including us (6–10), demonstrated that IL-5 enhances IgA formation by LPS-stimulated B cells. As we reported, IL-5 can stimulate DNP-primed $sIgA^+$ B cells for anti-DNP IgA antibody production (6). It is also evident from Table III that LPS-stimulated $sIgA^-$ B cells do not respond to IL-5. These results suggest that IL-5 predominantly acts on $sIgA^+$ but not $sIgA^-$ B cells as a maturation factor for IgA production. Taking in account the fact that IL-5 induces IgM and IgG1 production and TGF-$\beta$ inhibits this production, TGF-$\beta$ enhances IgA formation by different mechanisms than those mediated by IL-5.

There are at least two possibilities to account for the enhancing effect of TGF-$\beta$ on IgA production. First, TGF-$\beta$ may expand $sIgA^-$ B cells to switch to $sIgA^+$ B cells. In this case, TGF-$\beta$ can be thought of as an IgA-specific class-switching factor. Alternatively, TGF-$\beta$ may simply be expanding a specific subset of B cells or B cells at a particular stage of differentiation that are already programmed to switch to IgA. The fact that TGF-$\beta$ was effective in enhancing IgA formation in $sIgA^-$ B cells (Table III) may support the idea that the enhancement of IgA formation by TGF-$\beta$ is caused by expansion of B cells switching from $sIgA^-$ to $sIgA^+$ and also by post-switched B cells at a particular stage of differentiation and by induction of differentiation of these cells to IgA-forming cells. Kawanishi et al. (17) reported that T cells in Peyer's patches, but not in spleen, induce LPS-stimulated IgM-bearing B cells to switch to $sIgA^+$ B cells. TGF-$\beta$ may be one of the candidates for such an IgA-switching factor, however, it is not well characterized concerning TGF-$\beta$-producing T cell subsets. If TGF-$\beta$ could be produced by the same T cell subset that produces IL-5, sequential stimulation of T cells by microbial antigen may induce preferential production of TGF-$\beta$ and IL-5, which in turn induces $sIgA^-$ B cells to $sIgA^+$ B cells followed by differentiation into IgA-secreting cells.

In conclusion, although TGF-$\beta$ inhibits B cell proliferation, it selectively induces IgA production by $sIgA^-$ B cells as well as suppresses IgM and IgG1 production, and TGF-$\beta$ acts on B cells additively with IL-5 for IgA production. The experimental system described here should prove to be very useful in further analysis of the steps involved in T cell–dependent IgA production of murine B cells.

## Summary

Effects of transforming growth factor $\beta$ (TGF-$\beta$) on IgA production by LPS-stimulated B cells have been studied. TGF-$\beta$ itself could augment polyclonal IgA production in concomitant inhibition of polyclonal IgM and IgG1 production. Furthermore, TGF-$\beta$ and IL-5 additively augmented IgA production. TGF-$\beta$ exerted its activity early in the culture (by 2 d in a 5-d culture) and IL-5 was required late in the culture. Surface $IgA^-$ ($sIgA^-$) B cells responded to TGF-$\beta$ for the development of IgA-secreting cells. By contrast, $sIgA^+$ B cells, but not $sIgA^-$ B cells, responded to IL-5 for IgA production. These results suggest that TGF-$\beta$ has a differential role in the induction of IgA production from IL-5 on murine-activated B cells.

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