Brief Definitive Report

EXPRESSION OF TAC ANTIGEN ON ACTIVATED NORMAL HUMAN B CELLS

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Recent studies (1–4) have shown that anti-Tac monoclonal antibody recognizes the human receptor for interleukin 2 (IL-2), which is expressed on plasma membrane of T cells activated by antigens or lectins. Our initial experiments showed that the expression of Tac antigen was restricted predominantly to activated T cells (1). In a murine system, however, it was reported that IL-2 receptors were present at low levels on Thy-1-negative spleen cells activated with anti-Ig-plus factors when examined by radiolabeled human IL-2 (5). Moreover, very recently Korsmeyer et al. (6) demonstrated the expression of Tac antigen in eight cases of hairy cell leukemia that proved to be B cell lineage malignancies, suggesting the possibility that IL-2 receptor may be expressed at certain stages of normal B cell development.

In this study, we directly demonstrate the expression of Tac antigen on normal human B cells activated with *Staphylococcus aureus* Cowan I (SAC) by the method of two-color fluorescence analysis. In addition, we show here that SAC-activated B cells proliferate in response to immunoaffinity-purified IL-2 and that the proliferative response is completely inhibited by anti-Tac antibody that blocks the membrane binding and action of IL-2. We will discuss the possible involvement of an IL-2 receptor system in the B cell immune response.

Materials and Methods

Cell Separations. Peripheral blood mononuclear cells from normal volunteers were isolated by Ficoll-Paque (Pharmacia, Inc., Uppsala, Sweden) density gradient centrifugation. To obtain a B cell–enriched population, T cells and adherent cells were depleted (7). T cell depletion was accomplished by twice removing by Ficoll-Paque centrifugation the cells that rosetted with 2-aminoethylisothiouronium bromide hydrobromide (AET)-treated sheep erythrocytes (E). Adherent cells were removed by the adherence to plastic dishes for 60 min at 37°C. The resulting B cell–enriched population, used as B cells, contained <0.1% E-rosetting cells and 80–90% surface immunoglobulin (sIg)-positive cells, when stained by the fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fraction of goat anti-human Ig (Cappel Laboratories, Cochranville, PA). T cell–enriched populations, also obtained by twice centrifuging E-rosetting cells through Ficoll-Paque, contained >95% E-rosetting cells and <1% sIg-positive cells.

Cell Activation with SAC. B or T cells (4×10^6) suspended in 2 ml of RPMI 1640 medium containing 10% fetal calf serum (FCS) and 30 μg/ml of gentamycin were cultured

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with various indicated concentrations of SAC (Immusorbin; Wako Pure Chemical Industries, Osaka, Japan) in Falcon 2006 round-bottom tubes for 3 d at 37°C under 5% CO_2. Cultured cells were filtered through meshes (50 μm pore size) to remove large fragments of SAC that might interfere with flow cytofluorometry.

Anti-Tac Antibody and Detection of Tac Antigen on the Cell Surface. The IgG fraction of the antibody from hybridoma ascites was purified by the gel and DEAE cellulose chromatography. After pepsin digestion, the $F(ab')_2$ fraction was separated by gel filtration and protein A-Sepharose (Pharmacia, Inc.) affinity chromatography, followed by conjugation with FITC (Sigma Chemical Co., St. Louis, MO). For the direct staining of Tac antigen on the cell surface, cells (1×10^6) were incubated (4°C, 30 min) with a saturating concentration (10 μg/ml) of FITC-conjugated $F(ab')_2$ fraction of anti-Tac antibody (FITC-anti-Tac), and washed twice with Hanks' balanced salt solution containing 1 mg/ml of bovine serum albumin (BSA) and 0.1% sodium azide. All samples were analyzed by flow cytofluorometry (Spectrum III; Ortho Diagnostic Systems, Westwood, MA).

Two-Color Fluorescence Analysis (8). SAC-activated B cells were simultaneously stained with anti-Tac and anti-human Ig. B cells (1×10^6) were sequentially incubated at 4°C for 30 min (with thorough washing after each reagent) with 10 μg/ml of anti-Tac IgG, 10 μg/ml of biotinyl horse anti-mouse IgG (Vecter Laboratories, Inc., Burlingame, CA), 5 μl of phycoerythrin (PE)-conjugated avidin (Avidin PE; Becton Dickinson Monoclonal Center Inc., Mountain View, CA), and FITC-anti-human Ig. Samples were passed on Spectrum III using an argon ion laser at 488 nm excitation wavelength. Green fluorescence (515–530 nm wavelength) from FITC, and red (>630 nm) from PE were detected independently and displayed as dot plots in the logarithmic scale of fluorescence intensity.

Assay for B Cell Proliferation. IL-2, purified from culture supernatants of Jurkat cell line by an affinity column coupled with anti-IL-2 monoclonal antibody, was generously provided by Dr. K. A. Smith (9). B cell proliferation induced by IL-2 was determined by the method of Muraguchi and Fauci (7). Briefly, SAC-activated B cell blasts were prepared by culturing resting B cells (2×10^6/ml) with SAC (10^{-4} vol/vol) for 3 d. These blasts (5×10^4) in 200 μl of medium were cultured with a twofold dilution series of IL-2 in triplicate in a 96-well plate. B cell proliferation, as indicated by the incorporation of 0.5 μCi/well of [^{3}H]thymidine (2 Ci/mol; Amersham Japan, Tokyo), was measured during the last 16 h of a 72-h culture. In the experiments of the inhibition of IL-2-induced B cell proliferation by anti-Tac antibody, the antibody ($10–10^{-3}$ μg/ml) was added to the triplicated cultures of SAC-activated B cell blasts (5×10^4/well) plus 0.5 U/ml of IL-2. The percent inhibition was calculated by the following formula: Percent inhibition = $100 \times [1 - (\text{cpm experimental} - \text{cpm medium control})/(\text{cpm IL-2 control} - \text{cpm medium control})]$, where medium control is the culture with medium alone (3,069 ± 42 cpm) and IL-2 control is 12,443 ± 592 cpm.

Results and Discussion

Tac Expression of Activated Normal B Cells. To examine whether Tac antigen is expressed on activated B cells, freshly separated B cells (2×10^6/ml) were cultured for 3 d with SAC, a B cell mitogen (7), at various concentrations. Cultured B cells were stained with FITC-conjugated $F(ab')_2$ fraction of anti-Tac antibody to avoid the binding of anti-Tac to B cells via Fc receptors. As shown in Table I, the expression of Tac antigen was clearly observed on B cells activated by SAC at $>10^{-4}$ concentration (vol/vol), when analyzed by Spectrum III. In contrast, B cells cultured with medium alone did not express Tac antigen. Fig. 1*A* shows the representative fluorescence pattern of Tac antigen on B cells activated by 10^{-4} (vol/vol) of SAC, indicating the wide distribution of fluorescence intensity. Under the same conditions, however, normal T cells were not stimulated by SAC to express Tac antigen (<1%) (Fig. 1*B*).

TABLE I

Expression of Tac Antigen on SAC-activated B Cells

Cultured with:	Tac^+ Cells (mean ± SEM, $n = 5$)
	%
Medium alone	1.5 ± 0.5
SAC 10^{-3} (vol/vol)	22.7 ± 6.2
10^{-4}	19.2 ± 1.5
10^{-5}	3.7 ± 1.0
10^{-6}	3.2 ± 0.8

B cells were cultured with SAC for 3 d, then stained with the FITC-conjugated $F(ab')_2$ fraction of anti-Tac antibody.

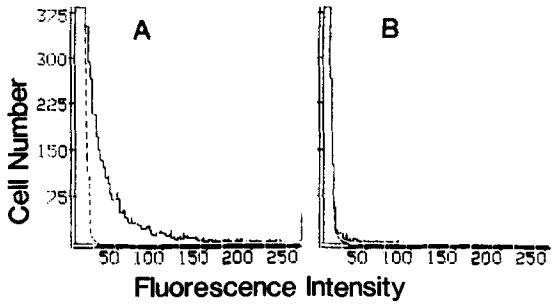

FIGURE 1. The fluorescence profile of Tac antigen on SAC-activated B cells (*A*) and T cells (*B*). Cells were cultured with SAC (10^{-4} vol/vol) for 3 d, stained by the FITC-conjugated $F(ab')_2$ fraction of anti-Tac antibody, and analyzed by the Spectrum III (linear scale). Dotted lines are nonstaining controls.

Two-color Fluorescence Analysis. Although T cell contamination in the B cell population was <0.1%, we performed two-color fluorescence analysis (8) to confirm that Tac antigen was expressed exactly on B cells, but not on T cells or non-T non-B cells. SAC-activated B cells were sequentially stained with anti-Tac, biotinyl anti-mouse IgG, and PE-conjugated avidin, followed by staining with FITC anti-human Ig. Green fluorescence from FITC and red from PE were independently detected by Spectrum III and displayed as dot plots. Fig. 2*D* clearly shows that Tac-expressing cells were included in the population of sIg-bearing cells. Fig. 2, *A*, *B*, and *C* shows the controls of nonstaining, single staining of Tac antigen, and single-staining of sIg, respectively.

An urgent question was then whether IL-2 generates any immune response of B cells by binding to Tac antigen/IL-2 receptor. We studied the proliferative response of SAC-activated B cells to affinity-purified IL-2. B cell blasts (5×10^4/well) activated by SAC for 3 d were cultured with serial dilutions of IL-2, and the proliferation of cells was determined after an additional 3 d of culture (7). As shown in Fig. 3*A*, SAC-activated B cells proliferated dose dependently in response to IL-2. In contrast, freshly separated B cells, which did not express Tac antigen, showed no detectable proliferation when cultured with IL-2 for 3 d, suggesting that the prior activation was required for IL-2-induced proliferation. These results are very similar to observations in IL-2-induced T cell proliferation (4). Expecting to find that IL-2-induced B cell proliferation is inhibited by anti-Tac antibody blocking the membrane binding and action of IL-

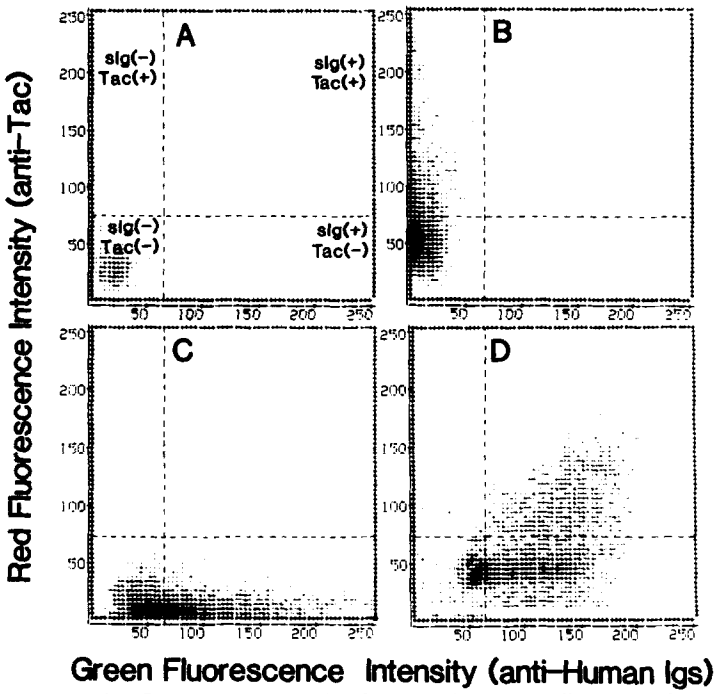

FIGURE 2. Two-color fluorescence analysis of SAC-activated B cells. (*D*) Both Tac antigen and sIg were stained as described in Materials and Methods. Tac^+ cells were included in the sIg^+ population. (*A*) Nonstaining control. (*B* and *C*) Controls of the single staining of Tac antigen and sIg, respectively.

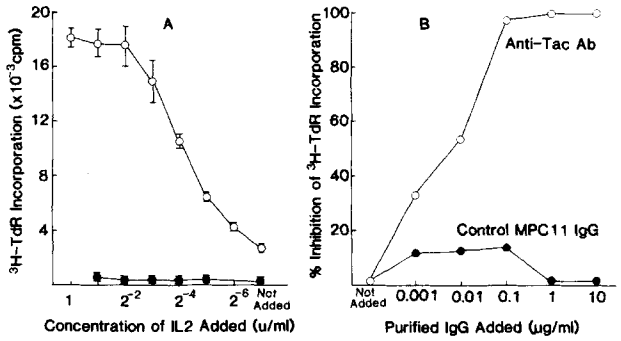

FIGURE 3. (*A*) Proliferative response of B cells to IL-2. SAC-activated B cells (5×10^4/well) (○) proliferated in response to IL-2, dose dependently, whereas freshly separated B cells (5×10^4/well) (●) showed no detectable proliferation. Data represent the mean ± SEM of triplicate cultures. (*B*) The inhibition of IL-2-induced B cell proliferation by anti-Tac antibody. SAC-activated B cells (5×10^4/well) were cultured in triplicate with 0.5 U/ml of IL-2 in the presence of anti-Tac (○) or control IgG (●) from MPC11 mouse myeloma cells. Data represent the percent inhibition of [^{3}H]thymidine incorporation.

2 in the T cell system, we cultured SAC-activated B cells with 0.5 U/ml of IL-2 in the presense of anti-Tac or control mouse myeloma (MPC11) IgG at various concentrations, as described in Materials and Methods. As shown in Fig. 3*B*, IL-2-induced B cell proliferation was remarkably inhibited by anti-Tac at very low concentrations. Similar inhibition was also observed in the experiments using

$F(ab')_2$ fragments of anti-Tac, indicating that the inhibition was not Fc receptor–mediated suppression.

A simple and reasonable explanation for our results may be that Tac antigen on SAC-activated B cells is the functioning IL-2 receptor, and that these B cells proliferate directly in response to IL-2. However, it is still controversial whether IL-2 is involved in B cell growth and/or differentiation. Some investigators (10, 11) have suggested that IL-2 itself plays an important role in the B cell growth, while Howard et al. (12) reported the possibility that IL-2 stimulates T cells to release, in turn, a B cell growth factor (BCGF) distinct from IL-2 which facilitates the proliferation of B cells. However, it is unlikely that in our experimental system the target cells of IL-2 were T cells and not SAC-activated, Tac-bearing B cells for the following reasons. First, the contamination of T cells in the SAC-activated B cell population was negligible (<0.5%) when examined by staining with FITC-OKT3 (data not shown). Second, the residual T cells, if any, did not express Tac antigen/IL-2 receptor when stimulated by SAC (Fig. 1*B*). Collectively, the data strongly suggest that IL-2 acts directly on SAC-activated B cells through the Tac antigen/IL-2 receptor. Tac antigen was not noted in our initial studies (1) on B cells activated with pokeweed mitogen (PWM) or Epstein-Barr virus, as measured by complement-dependent cytotoxity. Our preliminary experiments, however, showed that a proportion of B cells in peripheral blood mononuclear cells activated with PWM expressed Tac antigen weakly but distinctly, when examined by the more sensitive cytofluorometry after two-color staining (data not shown). Considering that anti-Tac at very low concentrations remarkably suppressed T cell–dependent B cell Ig production by PWM (13 and our unpublished data), the target cells of anti-Tac may be not only T cells but also B cells.

The mechanism of B cell growth and differentiation seems to be a complicated one in which many factors participate. Our studies indicate that an IL-2 receptor system also appears to be involved in B cell immunity.

Summary

Two-color fluorescence analysis revealed that Tac antigen, which was previously reported to be restricted to T cells, was expressed on a proportion of normal B cells activated by *Staphylococcus aureus* Cowan I (SAC). Immunoaffinity-purified interleukin 2 (IL-2) induced the proliferation of SAC-activated B cells, and the proliferation was completely inhibited by anti-Tac antibody, which blocked the membrane binding and action of IL-2. These results suggest that an IL-2 receptor system is directly involved in the B cell immune response.

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Note added in proof: Recent SDS-PAGE analysis of Tac antigen on SAC-activated B cells revealed the same band (60–65 kD) as that of PHA-activated T cells.

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