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...
with various indicated concentrations of SAC (Immosorbin; Wako Pure Chemical Industries, Osaka, Japan) in Falcon 2006 round-bottom tubes for 3 d at 37°C under 5% CO₂. Cultured cells were filtered through meshes (50 μm pore size) to remove large fragments of SAC that might interfere with flow cytometry.

**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 × 10⁶) 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 cytometry (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 × 10⁶) 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 (Vector 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 × 10⁶/ml) with SAC (10⁻⁴ vol/vol) for 3 d. These blasts (5 × 10⁴) 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 [³H]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 to 10⁻³ μg/ml) was added to the triplicated cultures of SAC-activated B cell blasts (5 × 10⁴/well) plus 0.5 U/ml of IL-2. The percent inhibition was calculated by the following formula: Percent inhibition = 100 \times \left\{1 - \frac{cpm \text{ experimental} - cpm \text{ medium control}}{cpm \text{ IL-2 control} - cpm \text{ medium control}}\right\}

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 × 10⁶/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. 1A 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. 1B).
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 10⁻⁴ (vol/vol) | 22.7 ± 6.2                  |
| 10⁻⁵          | 19.2 ± 1.5                    |
| 10⁻⁶          | 3.7 ± 1.0                     |
| 10⁻⁷          | 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.

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. 2D 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 x 10⁴/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. 3A, 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-
Green Fluorescence Intensity (anti-Human IgG)

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

FIGURE 3. (A) Proliferative response of B cells to IL-2. SAC-activated B cells (5 × 10⁴/well) (C) proliferated in response to IL-2, dose dependently, whereas freshly separated B cells (5 × 10⁴/well) (a) 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 × 10⁴/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 [³H]thymidine incorporation.

In the T cell system, we cultured SAC-activated B cells with 0.5 U/ml of IL-2 in the presence of anti-Tac or control mouse myeloma (MPC11) IgG at various concentrations, as described in Materials and Methods. As shown in Fig. 3B, 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. 1B). 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 cytotoxicity. 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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