Endotoxin-induced serum factor controlling differentiation of bone-marrow-derived lymphocytes

(induction/helper factor/antibody production)

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Communicated by Lewis Thomas, December 2, 1976

ABSTRACT Serum from bacillus Calmette-Guerin-infected mice injected with endotoxin induces the appearance of surface immunoglobulin, Ia antigen, and complement receptor on the surface of precursor bone-marrow-derived (B) cells. While endotoxin itself causes phenotypic conversion of both thymus-derived (T) cells and B cells in vitro, the endotoxin-induced serum factor was found to be a selective inducer of B cell differentiation. Spleen cells rendered immunodeficient by removal of B cells bearing the complement receptor regained the capacity to cooperate with helper T cells and to produce antibody against red cell antigens in vitro upon addition of the serum factor to the culture medium. Thus, a factor that controls selective phenotypic and functional differentiation of B cells has been identified and can now be characterized.

Lymphocyte differentiation proceeds through a sequence of steps which can be dissected by analyzing the expression of distinctive cell surface components. In the mouse, mature bone-marrow-derived (B) cells have been shown to carry Ig (immunoglobulin) (1, 2), Ia (I-region-controlled antigen) (3), complement receptors (CR) (4), Fc receptors (5) and PC antigens (6), whereas thymus-derived (T) cells characteristically exhibit Thy-1 (thymocyte alloantigen) (7) and Ly antigens (8-10) and also, where genetically appropriate, TL antigens (11). Expression of these markers on the surface of defined populations of precursor cells can be induced in vitro in an assay originally described by Komuro and Boyse (12). A number of agents induce the appearance of both B and T cell markers (13). Thymopoietin (14) acts more selectively; it induces differentiation of T cells but not of B cells (15). An agent which induces differentiation of B cells only has yet to be described for the mouse. In chickens, however, a factor isolated from the bursa by Brand et al. behaves like a selective B cell inducer (16).

We have reported recently that an endotoxin-induced serum factor in the mouse can replace helper T cells in the immune response to sheep erythrocytes (SRBC) in vitro (17). The factor is found in the serum of bacillus Calmette-Guerin (BCG)-infected mice injected with endotoxin (18); serum from normal mice, BCG-infected mice, or from normal mice injected with endotoxin is inactive. Interest in the properties of this serum was stimulated by our observation that it causes acute necrosis of a variety of transplanted tumors in the mouse (18). For this reason the serum has been designated tumor necrosis serum (TNS), and will be referred to as such throughout this report. The tumor necrotizing factor (TNF) of TNS migrates with α2-macroglobulins, and partial purification indicates that it is a glyco- protein with a molecular weight of approximately 150,000 (19).

We have now found that TNS induces structural and functional changes of B cells in vitro but does not induce differentiation of prothymocytes under conditions where thymopoietin is active.

MATERIALS AND METHODS

Mice. (C57BL/6 × A)F1 mice from our colony and (C57BL/6 × DBA/2)F1 (BDF1) female mice from Jackson Laboratory, Bar Harbor, Maine, were used. All mice were 3-4 weeks of age at the time of study.

Antisera—Anti-Ia*. A.TH mice were immunized with lymphoid tissues of A.TL mice. Hyperimmune serum was absorbed with (C57BL/6 × A)F1 thymus cells (13).

Anti-Ig. Antiserum to myeloma MOPC 104 E or to F(ab)2 fragments of myeloma MOPC 21 were raised in rabbits. Antibodies were isolated by absorption on IgM or IgG/F(ab)2 immunoadsorbants and subsequent acid elution (13).

Anti-Thy-1.2. (A/Thy-1.1 × AKR/H-2b)F1 mice were immunized with the A strain leukemia ASL1 (13).

Anti-SRBC. The 195 fraction of rabbit antiserum to SRBC was obtained from Cordis Laboratories (Miami, Fl.).

Induction Assays. These were performed, as described, for the following surface markers: Thy-1.2 (12), Ig (20), immune response associated antigen (Ia) (13), and CR (13).

Inducing Agents. Escherichia coli lipopolysaccharide (LPS) was provided by C. Galanos (Freiburg, Germany); calf-thymopoietin and synthetic thymopoietin by G. Goldstein of this Institute. TNS was prepared in CD-1 mice infected with BCG (Tice strain, Institute for Tuberculosis Research, University of Illinois Medical Center, Chicago, Ill.) by injecting 2 × 106 viable organisms intravenously. Fourteen days after BCG infection, mice were injected with 25 μg of endotoxin (lipopolysaccharide W from E. coli, Difco, Detroit, Mich.) intravenously and exsanguinated 2 hr later (18).

Enumeration of Cells Carrying Induced Surface Markers. Cells carrying Thy-1.2, Ig, or Ia were identified by assays of complement-dependent cytotoxicity (12, 21); cells carrying CR were assayed by the rosette assay (4).

Assays of Functional Activity. The culture system described by Mishell and Dutton was used (22).

Preparation of Columns. Sephadex G-10 columns were prepared as described by Ly and Mishell (23) for the removal of macrophages from mouse spleen cells. One volume of Sephadex G-10 was washed five times in five volumes of distilled water and twice in saline. Aliquots (30 ml) of Sephadex G-10 in saline were autoclaved at 110° for 30 min and stored at room temperature. Columns were prepared by loading sterile 10 ml plastic syringe barrels with 8 ml of Sephadex G-10, warmed up to 42°, and the Sephadex was washed with 20 ml of balanced...
salt solution (BSS), also at 42°. This column will be referred to as Seph column. Seph-Ag columns were prepared by incubating Seph columns with 5 ml of a solution of concanavalin A in BSS (1 mg/ml) for 10 min at room temperature. Con A that was not bound to Sephadex was eluted with 20 ml of BSS. Seph-Ag-Ab columns were prepared by incubating Seph-Ag columns with rabbit antiserum against Con A (diluted 1:20 in BSS) at room temperature, and washing them with 20 ml of BSS. Seph-Ag-Ab-C columns were prepared by incubating Seph-Ag-Ab columns with serum of BDF mice (diluted 1:10 in BSS) for 45 min at 37°. Before addition of spleen cells, each column was washed with 20 ml of BSS (42°), and 5 ml of BSS containing 5% fetal calf serum at 42°. Spleen cells in complete culture medium were kept on ice until used. One milliliter of the cell suspension (10^6 cells) was passed through the column, followed by 5 ml of BSS containing 5% fetal calf serum. The cells recovered in the eluate were washed once in medium before culturing (24).

RESULTS

Induction of lymphocyte surface markers by TNS and thymopoietin

Bone marrow cells [for induction of BAF, Ig, Ia, and Thy-1 (T cell marker) or spleen cells (for induction of CR) of BDF mice were separated on a discontinuous bovine serum albumin gradient (13, 25) and the cells of the C layer (the 25–36% in-
Influence of TNS on collaboration of B cells with primed helper T cells

We have shown that TNS (i) induces CR⁻ → CR⁺ conversion in spleen cells and (ii) enables CR⁻ spleen cells to produce antibody under conditions where they do not produce antibody in its absence. While there is no direct evidence that expression of CR is a prerequisite for antibody formation, the similar dose-response relation between TNS and augmented antibody production by CR⁻ spleen cells (Fig. 3) and between TNS and induced expression of CR, is compatible with this view. Further support comes from experiments, where cooperation between primed helper T cells and CR⁻ or CR⁺ spleen cells was measured. Spleen cells from mice immunized with SRBC, and depleted of CR⁺ cells (and macrophages) by passage through a Sephadex G-10 column coated with antibody/antigen/complement (24), were used as a source of primed helper T cells. One part SRBC-primed spleen cells was mixed with 9 parts unprimed CR⁺ or CR⁻ spleen cells, and the cultures were challenged with trinitrophenyl-conjugated SRBC (29). As shown in Fig. 4, addition of primed helper T cells resulted in considerably increased antibody production by CR⁺ spleen cells, but not by CR⁻ spleen cells. Addition of TNS and primed helper T cells increased greatly the response of CR⁻ spleen cells. In the absence of helper cells, TNS restores the response of CR⁻ spleen cells only to the level of the primary response observed with cultures of CR⁺ spleen cells (see also Fig. 2). The additional increase seen here in the presence of helper T cells suggests that TNS enables CR⁻ B cells to cooperate with them. Endotoxin had the same action on cultures of CR⁺ cells, but suppressed in the absence of normal mouse serum the response of CR⁺ spleen cells (17). As it has been shown that CR⁻ spleen cells are deficient in their capacity to cooperate with helper T cells (24), the results are consistent with the suggestion that the TNS-induced differentiation of CR⁻ → CR⁺ cells is the basis for the observed change in functional capacity.

DISCUSSION

Cell surface markers characteristic for B lymphocytes and T lymphocytes can be induced in precursor cells by a variety of agents (13, 15). A feature shared by all such agents is that they raise intracellular levels of cyclic AMP, and an increased ratio of cyclic AMP/cyclic GMP has been considered a signal of induction (30). To the extent that inducing agents act on surface receptors common to T and B lymphocyte precursors such as β-adrenergic receptors, they can be expected to induce phenotypic conversion of precursor cells belonging to both the T and B cell series. This has indeed been demonstrated for epinephrine, isoproterenol, and ubiquitin (15). Other inducing agents are more selective in their action. Thymopoietin, for example, induces differentiation of T cell precursors but not B cell precursors at subnanogram concentration (induction of B cell markers is seen only at much higher thymopoietin concentrations). TNS, on the other hand, appears to induce differentiation of B cell precursors but not T cell precursors. The selective action on B cell precursors argues against the possibility that residual LPS is the active principle (LPS induces phenotypic conversion of T and B cells at similar concentrations). Three B precursor subpopulations, characterized by the surface phenotypes Ig⁻IA⁻CR⁺, Ig⁺IA⁺CR⁻, and Ig⁺IA⁺CR⁺ acquire, on induction with TNS, Ig, Ia, or CR, respectively. They show decreasing sensitivity, in that order, to induction by TNS, and TNS does not induce the plasma cell antigen PC.1 in what are probably Ig⁺IA⁺CR⁺ precursor cells. As these inducible B cell subpopulations are thought to represent successive stages in B cell ontogeny (26) the failure to induce PC.1 may reflect the need for a unique inductive stimulus for this late differentiation step. The fact that TNS also substitutes for helper T cells (17) points to a possible connection between the signals imparted to B cells by helper T cells and the active principle in TNS—both promoting differentiation of B lymphocytes.

Induction of B cell markers by TNS was accompanied by functional maturation, measured in terms of antibody production in vitro. We showed previously that spleen cells depleted of CR⁺ B cells are deficient in their capacity to produce antibody to SRBC in Mishell-Dutton cultures, and we proposed that CR⁻ B cells must differentiate to CR⁺ B cells for optimal cooperation with helper T cells. This view is now supported by the finding that both TNS and LPS (which induce CR on the surface of CR⁻ B cells) restore the primary antibody response of CR⁻ spleen cells to SRBC, and permit the cooperation of CR⁻ B cells with primed helper T cells.

Whether CR merely marks a crucial stage in B cell differentiation, or represents a cell surface component essential for the cooperation of T and B cells, is at the moment unclear. It should be emphasized that the inability of CR⁻ spleen cells to produce antibody in vitro may well depend on the procedure and conditions used in the experiments described here. While some published studies are in agreement with our results as far as this deficiency of CR⁻ spleen cells is concerned (31, 32), others are not (33). In part, conflicting results may be due to differences in the methods that have been used for the purifi-
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cation of CR− cells. Comparative studies with CR− B cells obtained by various techniques are now in progress.

We have recently found that peritoneal macrophages from normal mice enable CR− spleen cells to cooperate with primed helper T cells (not with unprimed T cells), and that the supernatant of cultures of activated peritoneal macrophages (but not of nonactivated macrophages) supports, like TNS, the cooperation of CR− B cells with both primed and unprimed helper T cells (24). This points to the activated macrophage as a possible source of the active factor in TNS, and proliferation and activation of macrophages is in fact the most striking phenomenon during the priming phase for TNS-production (18). Some effects of TNF, on the other hand, mimic effects of factors assumed to be T cell derived (M. K. Hoffmann and U. Hammerling, unpublished observations). Current attempts to determine the cellular source of the factors that have now been defined in TNS, including (i) the B cell-inducing factor, (ii) the T cell helper factor, (iii) tumor necrosis factor (TNF), and (iv) the cytotoxic factor for tumor cells in vitro, and to ascertain whether these functional activities reside in a single molecule by chromatographic separation and other procedures have not succeeded.

This work was supported in part by grants from the National Institutes of Health (CA17673, CA16889, CA17085, and HO-08415), and by the American Cancer Society Grants (IM92 and IM97).


