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PRODUCTION AND CHARACTERIZATION OF A BOVINE T CELL-SPECIFIC MONOCLONAL ANTIBODY IDENTIFYING A MATURE DIFFERENTIATION ANTIGEN[1]

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A monoclonal antibody (MAb), BLT-1, with specificity for bovine mature T cells was prepared by somatic cell hybridization of myeloma NS-1 and spleen cells from BALB/c mice hyperimmunized with bovine T lymphocytes. The MAb reacted with over 92% of nylon wool-nonadherent lymphocytes (T cells) but not with nylon wool-adherent EAC-positive lymphocytes (B cells) in the indirect immunofluorescence assay. It is an IgM, with κ-light chains, which fixed complement well and killed over 95% of mature T cells in complement-mediated cytotoxicity assays. It reacted with the same proportions of peripheral lymphoid cells (peripheral blood, lymph nodes, and spleen) as the polyclonal goat anti-bovine thymocyte serum (GABTS), but only with 25% of GABTS-positive thymocytes. Immunoperoxidase staining of frozen tissue sections showed that the BLT-1-positive cells were located in the medulla of the thymus and in the T lymphocyte areas of lymph nodes. Western immunoblotting assays showed that the BLT-1-reactive membrane antigen is a 22,000 m.w. protein which was inducible in bovine thymocytes with bovine thymic hormones, thymosin fraction 5, thymosin α_1, and thymopentin ORF-18150, indicating that it is a mature T lymphocyte differentiation antigen. The thymosin α_1 and thymopentin were found to show additive effects on mature T cell antigen expression by bovine thymocytes.

The study of the bovine immune system, in addition to its economically important contributions to disease prevention and control, has had significant impact on basic immunology. For example, studies of calf dizygotic twins (1, 2) have opened up the field of immunologic tolerance research, and bovine-derived terminal deoxynucleotidyl transferase (3) and thymic hormones (4) have proved invaluable in the understanding of T lymphocyte differentiation and maturation.

In this communication, we report on the production and characterization of a monoclonal antibody (MAb),[3] bovine lymphocyte T-1 (BLT-1), which specifically reacts with a bovine mature T cell antigen. Induction of the T cell differentiation antigen in thymocytes by thymic hormones from the homologous species supplements and extends the previous findings on the effects of bovine-derived thymic hormones on human, mouse, and rat lymphoid cells (5–7).

MATERIALS AND METHODS

Animals/tissues. BALB/c mice, bred and maintained at the University of Connecticut, Department of Pathobiology, were used in MAb preparation. Bovine blood was obtained from adult Holstein-Friesian, Jersey, and Guernsey cows at the University of Connecticut Dairy Farm, and Holstein lymph nodes and thymuses were obtained from a local slaughterhouse (Home-Pride Provisions, Inc., Stafford Springs, CT).

Hybridomas. Female BALB/c mice, 10 to 12 wk of age, were immunized with three i.v. injections of 10^7 bovine T cells (as processed below) at weekly intervals. Spleens were harvested after the final injection, and 9×10^7 cells were mixed with 3×10^7 P3/NS-1/1-Ag4-1 myeloma cells. After centrifugation for 5 min at 250 × G, the cells in the pellet were suspended in 1 ml of 50% polyethylene glycol 1000 (Koch-Light Laboratories, Coinbrook, Bucks, UK) in serum-free Dulbecco's minimal essential medium (DMEM; GIBCO, Grand Island, NY) for 1 min, were gradually diluted, and were then washed and resuspended in 60 ml DMEM-20% fetal calf serum (FCS). After incubation overnight at 37°C in a CO_2 incubator, the cells were washed and were resuspended in 140 ml HAT medium (8) containing 6×10^6 BALB/c thymocytes as feeder cells (9), and 200-μl aliquots of the cell suspension were dispensed into the wells of 96-well plates and were incubated at 37°C in a CO_2 incubator.

Ten to 14 days after the cell fusion, wells with positive growth were screened for anti-bovine T cell antibody by employing the whole cell enzyme-linked immunosorbent assay (ELISA; described below). Positive hybridomas were cloned by limiting dilution, using BALB/c thymocytes as feeders. Ascites were produced by i.p. injection of 1 to 5×10^6 cloned and recloned hybridoma cells in pristane-primed syngeneic mice.

The immunoglobulin (Ig) isotypes and subclasses were determined by the ELISA technique employing heavy chain- and light chain-specific rabbit antisera (Mono Ab-ID EIA kit; Zymed Laboratories, San Francisco, CA).

Peripheral blood lymphocytes (PBL). Six milliliters of heparinized bovine blood were diluted with 3 ml of calcium- and magnesium-free phosphate (0.01 M)-buffered saline (PBS), pH 7.2, and were placed over 3 ml of Ficoll Diatrizoate (F-D; density = 1.084 g/cc, Ficoll Type 400; Sigma Chemical Co., St. Louis, MO; Diatrizoate sodium; Sterling Drug Co., New York, NY). After centrifugation at 400 × G for 45 min, the peripheral blood mononuclear cells (PMC) at the interface were harvested, were washed, and were treated with Tris-buffered NH_4Cl for 1 min at room temperature to lyse contaminated erythrocytes (RBC). They were washed twice in PBS and were resuspended in RPMI 1640 medium containing 10 mM glucose, 25 mM HEPES, 2

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[3] Abbreviations used in this paper: MAb, monoclonal antibody; DRBC, dog erythrocytes; TBS, Tris-buffered saline; B-HAM IgG, biotinylated horse anti-mouse IgG; NS-1, P3/NS-1/1-Ag4-1 myeloma cells; C, complement; DMEM, Dulbecco's minimal essential medium; PBL, peripheral blood lymphocytes; PMC, peripheral blood mononuclear cells; F-D, Ficoll-Diatrizoate; IF, immunofluorescence; EAC, erythrocyte-antibody-complement; BLT-1, bovine lymphocyte T-1; GABTS, goat anti-bovine thymocyte serum.