CD5\(^+\) B Cells Are Decreased in Peripheral Blood of Patients with Crohn’s Disease

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B cells bearing the CD5 surface marker comprise a substantial minority of the circulating lymphocyte population in healthy individuals. These recently described cells have been implicated in T-independent humoral responses, immunoregulation, and autoimmunity. We undertook to enumerate circulating CD5\(^+\) B cells by three-color fluorescence activated flow cytometry in 28 patients with Crohn’s disease (CD). None of the CD patients were using immunosuppressive medication. The CD patients were subdivided into “inactive” and “active” groups based upon their Crohn’s disease activity index (CDAI). Thirty-two normal subjects served as a control population. The percentage of CD19\(^+\) B cells was significantly reduced in both active and inactive CD patients as compared with normal controls (P < 0.01). CD5\(^+\) B cells were likewise found to be significantly decreased in both inactive and active CD patients (P < 0.01) as compared with normal controls. The proportion of CD5\(^+\) B cells was significantly lower in the peripheral blood of active as compared with inactive CD patients (P < 0.05). The finding that CD5\(^+\) B cells are reduced in CD may provide an important clue to immunological dysfunction in inflammatory bowel disease and merits further study.

KEY WORDS: CD5 surface marker; B cells; Crohn’s disease.
CD5+ CELLS IN CROHN'S DISEASE

While the precise function of CD5+ B cells has not been elucidated, it has been suggested that their characteristics and distribution suit them to a primary defensive role against bacterial pathogens. They may likewise function in "primitive" humoral immune responses where T-cell help is not available or inadequate (19) CD5+ B cells may also serve an immunoregulatory function by secreting antidiotype antibodies that down-regulate or eliminate specific B-cell clones (22, 23). Several observations have also linked CD5+ B cells to some autoimmune processes (24, 25). For example, CD5+ B cells are increased in the peripheral blood of patients with rheumatoid arthritis (25), systemic lupus erythematosus (26), and Sjogren's syndrome (27) but not in those with Grave's disease (25). Because of the possible role of CD5+ B cells in autoimmune disease and immunoregulatory processes, we undertook to enumerate CD5+ B cells in patients with CD using three-color flow cytometry.

MATERIALS AND METHODS

This study was reviewed and approved by the University of Iowa Human Studies Committee.

Control Subjects. Thirty-two normal control subjects ranging in age from 21–57 years (mean age 32.1 ± 4.2 years) were recruited from the staff and students of the University of Iowa. Nineteen nonpregnant females and 13 males participated in the study. All subjects denied any history of significant illness or the use of immunosuppressive medications.

Crohn's Disease Subjects. Twenty-eight patients with biopsy-proven Crohn's disease were recruited for the study. The sample included 13 nonpregnant females and 15 males ranging in age from 21 to 70 years (mean age 37.4 years). Pertinent clinical data were collected from each patient including duration and extent of their disease, current medications, and other information to allow calculation of the Crohn's disease activity index (CDAI) for each patient (28). None of the patients had used corticosteroids, azathioprine, 6-mercaptopurine, metronidazole, or other immunosuppressive drugs for at least one month prior to study. A summary of the CD subjects is presented in Table I.

For the purposes of statistical analysis, the CD patients were divided into "inactive" and "active" groups according to their CDAI. Inactive patients were defined as having a CDAI of 100 or less (18 subjects, CDAI = 98.6 ± 34.8). Active subjects were defined as having a CDAI of >100 (10 subjects, mean CDAI = 183.6 ± 37.1).

Cell Isolation and Preparation. Seven milliliters of blood was collected in heparinized tubes by venipuncture from each of the control and CD subjects. The mononuclear cell fraction was prepared by centrifuging the blood through Ficoll–Hypaque, specific gravity 1.077 (Sigma, St. Louis, Missouri). The cells were then washed in cold flow cytometry (FACS) buffer (balanced salt solution supplemented with 5% fetal calf serum and 0.1% NaN₃). After counting, aliquots of 5 × 10⁶ cells were dispensed into 1.5-ml tubes, centrifuged, and resuspended in 15 μl of FACS buffer for staining with specific murine MAb as given below.

Cell Staining. The processed cells were stained in a two-step procedure wherein the cells were initially incubated for 20 min at 4°C with a cyanoine 5.18 conjugated anti-B cell antibody, anti-CD19 (4G7), biotin-conjugated anti-CD5 (L17 F12), and fluorescein isothiocyanate-conjugated anti-HLA-DR (Becton Dickinson, Mountain View, California). After washing, the cells were further incubated with phycoerythrin-labeled avidin (Fisher Biotech, Pittsburgh, Pennsylvania). Subsequent to final washing, the cells were fixed in 2% paraformaldehyde and kept in the dark until analyzed. Chromatographically purified mouse IgG (Cal Biochem, La Jolla, California) conjugated to cyanoine 5.18, fluorescein isothiocyanate, and biotin served as isotype controls for each analysis.

FACS Analysis. Stained cells were analyzed in the University of Iowa Pathology Flow Cytometry Facility using a modified Becton-Dickinson FACS 440 equipped with four decade logarithmic amplifiers. In addition to the primary argon ion laser, the instrument was equipped with a rhodamine 6G CR599 dye head laser (Coherent, Palo Alto, California) pumped with a secondary argon