IMMUNOLOGICAL STUDIES ON CROHN'S DISEASE
V. ENUMERATION OF CIRCULATING LYMPHOCYTES SUBSETS USING MONOCLONAL ANTIBODIES

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Summary

We found normal levels of suppressor cell activity and reduced natural killer (NK) and antibody dependent cell-mediated cytotoxicity (ADCC) activities in patients with Crohn's disease (CD). To further characterize these activities, studies were carried out using monoclonal antibodies. There were no changes in the proportion of OKT4+ (helper/inducer T cells), OKT8+ or Leu 2+ (suppressor/cytotoxic T cells) and Leu 7+ cells (large granular lymphocytes: LGL, NK + K cells), thereby suggesting that suppressor cell activity in CD is likely to be normal both in function and in number, and that depressed NK and ADCC activities are not due to a reduction in the number of NK or K cells but rather to functional defects. Using a double staining method, we noted a low percentage of both Leu 2 and Leu 7 positive cells in CD.

Key Words: Crohn's disease, lymphocyte subsets, monoclonal antibodies.

Introduction

The availability of monoclonal antibodies (MAb) that selectively bind to lymphocyte subsets has facilitated evaluation of immune status in health and in disease1). The use of a flow cytometer to distinguish lymphocytes from other leucocyte types enables a simple and rapid measurement of lymphocytes reactive with MAb2). Elucidation of the variability of lymphocyte subsets, especially T cell subsets, in the peripheral blood is vital to correctly interpret the findings in various disease states. Thus, it has been shown that there is a decreased proportion of total T cells or suppressor T cells in autoimmune diseases such as systemic lupus erythematosus3), Sjögren's syndrome4), multiple sclerosis5) and autoimmune thyroid diseases6). Such a depressed number of suppressor T cells may be related to disease activity and the role of immunological abnormalities in the pathogenesis has to be given attention.
We examined the proportions of helper and suppressor T cells and NK + K cells, as defined by MAbs, in the peripheral blood of patients with Crohn's disease (CD), and comparison were made with findings in our previous studies\(^7^-^9\).

**Materials and Methods**

**Subjects**

Heparinized peripheral blood samples were obtained from 16 Japanese patients with CD at Fukuoka University Hospital and 16 healthy controls. The severity of disease was classified according to the criteria of Best et al.\(^10\).

**Lymphocyte preparation**

Mononuclear cells were isolated by Ficoll-Conray density gradient centrifugation and were washed with phosphate-buffered saline (PBS). Cells were resuspended in HEPES-buffered RPMI 1640 (GIBCO, U.S.A.) supplemented with 10% heat-inactivated fetal calf serum, 100 μg/ml penicillin G and 100 μg/ml streptomycin.

**Monoclonal antibodies**

Anti-Leu 2 (Becton & Dickinson, U.S.A.) and OKT8 (Ortho Diagnostic System, U.S.A.) antibodies are reactive with peripheral T cells involved in cell-mediated cytotoxicity and suppression. OKT4 identifies lymphocytes that function primarily as helper and inducer cells. Anti-Leu 7 antibody (HNK-1) is reactive with the large granular lymphocytes which include NK and K cells.

**Quantitation of lymphocyte subsets**

For indirect immunofluorescence studies, OKT4 and OKT8 were used. After washing, lymphocytes were incubated with appropriate monoclonal antibodies for 10 min at room temperature. The cells were incubated for 30 min at 4°C with a fluorescein isothiocyanate (FITC)-conjugated goat antimouse immunoglobulin. Ten thousands cells were analyzed by flow cytometry using a fluorescence-activated cell sorter (FACS: EPICS V, Coulter Electronics, U.S.A.).

**Double labeling study**

Direct immunofluorescence assay was used for the double staining. For two-colour staining, \(10^6\) target cells were added to the antibody mixture (FITC-labelled Leu 2 and phycoerythrin-labelled Leu 7) at a concentration of \(10^6\) per \(100 \mu l\). After 30 min of incubation on ice, these cells were washed three times. Two-color immunofluorescence analysis was performed with a FACS II (Becton & Dickinson, U.S.A.) equipped with an argon ion laser producing 200 MW at 488 nm. During the analysis, we used a PC computer (PC-9801E, NEC Computer System, Japan) to collect and store individual measurements on 100,000 cells at list mode data for later analysis.

**Statistical analysis**

Student's t-test was used and a p<0.05 was considered to be of statistical significance. Person's correlation coefficient test was also used.

**Results**

Using monoclonal antibodies, the OKT series, variations in the percentage of peripheral blood T lymphocyte subpopulations (OKT4 & OKT8) were assayed (Table 1). There were no significant differences in the proportion of OKT4+ cells and OKT8+ cells, nor in the OKT4+/OKT8+ ratio between patients with CD and healthy controls. In some patients the OKT4+/OKT8+ ratio exceeded that of the controls. There was no relationship between the proportion of T cell subsets and disease activity.

In other studies, Leu series, the number of suppressor T cells was evaluated and much the same findings were obtained (Table 2). As for killing system against microbial agents, we already reported decreases in both NK and ADCC activities\(^8^-^9\). The cell number bound to...