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ALTERATION OF T-LYMPHOCYTE SUBPOPULATIONS IN SUBACUTE AND CHRONIC BRUCELLOSIS

A.A. POURFATHOLLAH, M.B. ESLAMI*, A. R. YALDA** AND A.F. SARRAF NEJAT*

From the Dept. of Immunology, School of Medical Sciences, Tarbiat Modarress University, Tehran, and the Departments of Pathobiology and **Infectious Diseases, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.*

ABSTRACT

Human brucellosis is a major health problem in developing countries. A number of patients with human brucellosis do not recover from the acute stage of the disease and pass into the chronic form. Since the pathophysiology of this change is not well understood, we studied the T-cell subsets during the acute, subacute and chronic forms of human brucellosis. In this study we found alterations in T-lymphocyte subsets and in the CD_4/CD_8 ratio in subacute and chronic brucellosis while no significant change was found in T-cell subsets in acute brucellosis. We believe that this alteration of T-cell subsets is important in the pathophysiology of brucellosis, but it is not clear whether brucella cause this change or a primary immunodysfunction in patients with a disturbance in T-cell subset regulation prevents recovery from acute brucellosis and leads to the development of chronic brucellosis.

Keywords: Brucellosis, CD_4, CD_8, T-lymphocyte, Alkaline phosphatase anti-alkaline phosphatase (APAAP) method.

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INTRODUCTION

Brucellosis is an important public health problem that occurs worldwide. It causes significant economic losses among domesticated farm animals and is frequently transmitted from animals to man in areas where the disease is enzootic.[1] There is a great deal of information regarding the humoral immune response against brucella[2-4] but less is known about the role of cell-mediated immunity in brucellosis. Since brucella are intracellular pathogens, cell-mediated immunity plays an important role in elimination of intracellular pathogens and development of a protective immunity against these microorganisms. In the present study, we investigated CD_3, CD_4 and CD_8 blood lymphocytes in acute, subacute and chronic forms of human brucellosis.

MATERIALS AND METHODS

Patients

56 patients who fulfilled the following criteria were included in this study: 1) clinical presentation compatible with brucellosis, and 2) brucella isolation or a serological titer of ≥ 1:160 agglutinin antibodies. On the basis of duration of clinical symptoms, the cases were classified arbitrarily as acute (less than eight weeks), subacute (from eight to 52 weeks) or chronic

(more than one year).[5]

Controls

Control blood samples were taken from 26 normal subjects, 15 males and 11 females, with a mean age of 31 years (range 23 to 55 years).

Bacteriological method

Before initiating antibiotic therapy a blood culture was performed in Ruiz-Casteneda medium for each patient by inoculating 5-10mL of peripheral blood into 35 mL of the medium. Cultures were incubated at 37°C for a minimum of 3 weeks, during which they were observed after 24, 48, and 72 hours and on the 4th, 7th, 14th, and 21st day after the initial inoculation. When any positive culture was found, a subculture for identification of the organism was attempted.[6,7]

Serological methods

Brucella abortus antigen was purchased from Pasteur Institute of Iran. This antigen is prepared according to the World Health Organization procedure for use in tube agglutination tests. Two-fold serial dilutions of the patients' sera were prepared starting with a serum dilution of 1/20, and 0.5 mL of *Brucella abortus* antigen was added in each tube. End point titers were read 18h after incubation at 37°C.[7] 2-ME and Coombs' agglutinations were carried out whenever necessary.

Lymphocyte subsets

Whole blood samples were drawn in heparinized tubes and separation of lymphocytes was carried out by Ficoll-Hypaque (Histopaque-1077, Sigma) after which three smears were prepared using a cytospinner (Cytospin 3, Shandone). The smears were air-dried and fixed for 60 seconds in acetone methanol (1:1 V/V), wrapped in aluminium foil and stored in -20°C until immunocytochemical staining. For immunocytochemical staining and determination of CD_3, CD_4 and CD_8 T-cells, we used the alkaline phosphatase anti-alkaline phosphatase antibody (DAKO Corp., Carpineria, U.S.A.) technique. Briefly, 3 steps of staining, incubation periods and washings were contemplated and 3 cell smears were layered with mouse monoclonal antibody against CD_3, CD_4 and CD_8. Then a second layer of rabbit antiserum to mouse immunoglobulins was added and finally alkaline phosphatase anti-alkaline phosphatase immune complex was added. Each incubation step lasted 3 min and washing was carried out in tris buffer (pH = 7.6) for 5 min. The alkaline phosphatase reaction was performed with naphtol As-MX and Fast Red TR salt in tris buffer and counterstained in Mayer's hematoxylin and the slides were mounted in glycerol gelatin while still wet for microscopic study.

For each smear 200 cells were counted and the percentage of lymphocytes positive and negative for CD_3, CD_4 and CD_8 antigens were recorded.[9]

Statistical analysis

The statistical analysis used in this study was Student's t-test.

RESULTS

The results of total white blood cell counts, lymphocyte counts, percentages of CD_3, CD_4, and CD_8 positive cells, and CD_4/CD_8 ratios from normal controls and acute, subacute and chronic patients are shown in Table I . As depicted, the results presented are statistically significant in chronic and subacute forms of the disease.

DISCUSSION

It is now well established that cell-mediated immunity (CMI) is mediated by thymus-dependent or

Table I. The absolute number/percentage of WBCs, lymphocytes, CD_3 , CD_4 , CD_8 and CD_4/ CD_8 ratio in brucellosis.

Patient group	Number	WBC/mL of blood	Lymphocytes/mL of blood	CD_3 %	CD_4 %	CD_8 %	CD_4/CD_8 ratio
Normal	26	4657±1421	1921±543	74±8	50±7	26±5	2.03±0.493
Acute brucellosis	31	6896±2542	2547±1225	73±8	47±7	26±5	1.86±0.528
Subacute brucellosis	15	6900±2271	2769±1098	68±7	36±8	33±8	1.160±0.491
Chronic brucellosis	10	7550±2434	2703±909	74±6	36±9	39±11	1.038±0.480

T-lymphocytes, and that within the T-cell population are functional subsets including those that provide both positive (help) and negative (suppression) regulation of the immune response. CD_4 lymphocytes make up the subset referred to as helper or helper/inducer T-cells, due to their ability to augment B-cell responses and to amplify the cell-mediated responses effected by CD_8 T-cells. Under certain circumstances CD_4 T-cells can also mediate cytotoxicity and immune suppression. CD_8 T-cells mediate most antigen-specific cytotoxicity.[10]

The results of the present study show an alteration in the number of T-cell subpopulations in subacute and chronic brucellosis. This change was not accompanied by changes in total lymphocyte counts. It is very likely that this change in subacute and chronic brucellosis is due to the persistence of pathogenic organisms in the body which brings about a continuous antigenic stimulation of the immune system, thereby causing a change in regulation of immune responses. Normally, initial stimulation of the immune system by an antigen leads to clonal expansion of the commited cells and the production of effector molecules which are under regulatory mechanisms. Upon continuous antigenic stimulation for a long period, a modulation of regulatory mechanisms occurs with a change in T-cell subpopulations and lymphokine production. A change in the normal ratio of CD_4 and CD_8 has been found in lepromatous leprosy and advanced tuberculosis.[11,12] A few cases of acute brucellosis do not recover and develop into chronic and subacute brucellosis. Genetic constitution may be associated with the progression of acute brucellosis to subacute and chronic forms of the disease. Hodinka et al.[13] reported an association of HLA-B27 with chronic brucellosis, as HLA-B27 was found in 12.8% of healthy Hungarians and in 25% of patients with chronic brucellosis.

Studies on T-cell clones derived from patients with infectious diseases such as leprosy have allowed the delineation of functional human T-cell subsets. Both CD_4 and CD_8 cells can be discriminated into subsets that are differentiated by their functions and patterns of lymphokines.[14] The lymphokine patterns may reflect differences of host response in acute, subacute and chronic brucellosis. The CD_8 T-cell is a minor source of IFN-γ.[15] Since in infections due to intracellular pathogens, lymphokines mediate killing of pathogens by macrophages, the increase of CD_8 cells may reflect an ineffective host response. CD_8 cells can be weakly protective in an adoptive transfer assay in tuberculosis and in our experience, in the sera of acute, subacute and chronic brucellosis patients, a significant decrease of γ-IFN was found in subacute and chronic forms in comparison to the acute form of the disease.[17]

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