Subpopulations of Peripheral Lymphocytes in Juvenile Diabetes

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SUMMARY

Subpopulations of peripheral lymphocytes were studied in 26 children with insulin-treated juvenile diabetes and in 27 control children of comparable age. T-lymphocytes were quantitated by spontaneous rosette-formation with sheep erythrocytes and B-lymphocytes by indirect immunofluorescence with the use of monovalent, fluorescein-labeled rabbit antiserum specific to the heavy chains of human IgG, IgM, or IgA. No significant quantitative difference in subpopulations of the peripheral lymphocytes, T-cells, and B-cells with IgG, IgA, or IgM markers was found between children with juvenile diabetes and the control group, although the B-lymphocytes with IgG or IgA markers tended to be higher and those with IgM markers lower in the diabetic than in the control group. DIABETES *25*:101-03, February, 1976.

The factors leading to the development of juvenile diabetes have not been completely clarified despite numerous attempts to implicate a variety of potential causes. There is, however, increasing evidence to implicate autoimmune mechanisms in its pathogenesis. Circulating antibodies to thyroid[1-4] and adrenal[2-3] tissues, gastric parietal cells,[2,4,5] intrinsic factor,[5] and finally to pancreatic islet cells[6-9] have been detected in patients with juvenile diabetes. In addition, evidence has emerged from in-vitro lymphocyte studies that suggests the involvement of cellular immunity in juvenile diabetes; lymphocytes from patients with juvenile diabetes have been shown to be sensitized to pancreatic tissue antigens[10,11] as well as to insulins of bovine and porcine origin.[12]

Recently, alterations in subpopulations of lymphocytes have been reported in a variety of immunodeficiencies,[13,14] lymphoproliferative disorders,[15,16] and autoimmune diseases.[17-19] Human lymphocytes consist of a heterogeneous population of cells with regard to origin, function, and stage of differentiation.[20] The thymus-dependent lymphocytes (T-cells) are primarily responsible for cell-mediated immunity and comprise the majority of the circulating lymphocytes. The rest are B-lymphocytes, which are thymus-independent and mainly responsible for humoral immunity. B-cells carry readily detectable surface immunoglobulin markers and, thus, can be further differentiated by the class of immunoglobulin markers that they bear.

In the present study, we have undertaken quantitation of subpopulations of peripheral lymphocytes with the use of the spontaneous rosette-forming and indirect immunofluorescent technics in order to determine whether or not there is any alteration in lymphocyte subpopulations in young patients with juvenile diabetes.

PATIENTS AND METHODS

Twenty-six patients with insulin-dependent diabetes of juvenile onset, three to 19 years of age, were included in this study. All of them had the diagnosis made at least six months previously and had been receiving insulin regularly to bring it under adequate control at the time of this study. The controls consisted of 27 children of similar age, 15 of whom were under routine health supervision in the outpatient clinic, seven children with mild to moderate obesity, and five volunteer youngsters. None of the controls had either chemical or clinical diabetes.

Preparation of lymphocyte suspension. Heparinized venous blood was collected at mid-morning or early afternoon. A portion of each sample was used for total and differential white blood cell counts. Lymphocytes were separated by density centrifugation on a Ficoll-metrizoate gradient,[21] and washed three times in McCoy's 5a medium (Microbiological Associates, Bethesda, Md.) and suspended in it. The final cell suspensions were adjusted to 1×10^6 per milliliter and contained more than 95 per cent lymphocytes with greater than 98 per cent viability by trypan blue exclusion.

Identification and enumeration of T-lymphocytes. The rosette-forming technic of Jondal and associates[22] was employed with slight modification. Fresh sheep eryth-

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rocytes supplied in Alsever's solution were washed and adjusted to a 1 per cent suspension in McCoy's 5a medium. To each of 0.25 ml. of lymphocyte suspension containing approximately 2.5 × 10^5 cells, 0.25 ml. of 1 per cent sheep erythrocyte suspension was added. The mixture was incubated for 10 min. at 37° C. in 5 per cent CO_2, centrifuged at 200 × G for five minutes and refrigerated for two to 15 hours. Lymphocytes attached by three or more sheep erythrocytes were taken as rosettes, and at least 200 of such cells were counted in a hemacytometer to determine the percentage of T cells.

Identification and enumeration of B-lymphocytes. Indirect immunofluorescence technic of Grey and associates[23] was used with the following modification. To each 0.1 ml. of lymphocyte suspension containing 5 × 10^5 lymphocytes, 0.1 ml. of 1:4 dilution of monovalent, fluorescein-labeled rabbit antiserum specific to the heavy chains of human IgG, IgM, or IgA (Behring Diagnostic, Somerville, N.J.) was added. The mixtures were incubated for 45 minutes at room temperature with occasional, gentle shaking, and then were washed three times and suspended in McCoy's 5a medium. At least 100 cells were counted for the determination of each subpopulation of B-lymphocytes. The monocytes and granulocytes were carefully eliminated from the count on the basis of morphology.

RESULTS

Results of the determinations of total peripheral lymphocytes, T-cells, and B-cells with their subpopulations in children with juvenile diabetes and control subjects are presented in tables 1 and 2. No statistically significant difference in the absolute numbers and percentages of these lymphocyte subpopulations is found between the two groups at p value of 0.05 by the Mann-Whitney *U* test.[24] The sex and age of the patients, the duration of the disease, and the length of insulin therapy do not seem to have affected the quantities of lymphocyte subpopulations in these patients. However, B-lymphocytes tended to be slightly higher in number in older diabetic children over 14 years of age than in the controls of comparable age. Also, B-lymphocytes with IgG and IgA markers tended to be higher while those with IgM marker lower in the diabetics in general.

TABLE 1

Absolute numbers and percentages of peripheral lymphocytes and their subpopulations in 26 patients with juvenile diabetes

Subpopulations of lymphocytes	Absolute number (count/mm.^{3}) Mean ± S.D.	Percentage Mean ± S.D.
Total lymphocytes	2,283 ± 591	100.00 ± 0.00
T-lymphocytes	1,431 ± 384	62.92 ± 5.09
B-lymphocytes		
Total	506 ± 299	21.52 ± 8.56
With IgG marker	320 ± 194	13.73 ± 6.18
With IgA marker	124 ± 99	5.19 ± 3.38
With IgM marker	62 ± 50	2.60 ± 1.53

TABLE 2

Absolute numbers and percentages of peripheral lymphocytes and their subpopulations in 27 control children

Subpopulations of lymphocytes	Absolute number (count/mm.^{3}) Mean ± S.D.	Percentage Mean ± S.D.
Total lymphocytes	2,269 ± 413	100.00 ± 0.00
T-lymphocytes	1,429 ± 264	63.14 ± 5.09
B-lymphocytes		
Total	464 ± 229	21.00 ± 9.00
With IgG marker	289 ± 132	12.74 ± 5.42
With IgA marker	97 ± 74	4.51 ± 2.99
With IgM marker	78 ± 54	3.75 ± 2.94

DISCUSSION

As previously stated, juvenile diabetes presents many features that suggest participation of immune mechanisms in its pathogenesis.[1-12] Therefore, it seemed important to us to quantify the subpopulations of the peripheral lymphocytes. The results of our study, however, show that there is no significant difference in the percentages and the absolute numbers of total lymphocytes, T-cells, and B-cells between children with juvenile diabetes and the control youngsters. The results are in agreement with those of MacCuish and associates,[25] in which the test group consisted mainly of adult patients with insulin-dependent diabetes. In the present study, the subpopulations of B-lymphocytes were further quantified by indirect immunofluorescent technic with the use of monospecific antiserum to the heavy chains of human immunoglobulin IgG, IgA, or IgM. B-cells with IgG or IgA markers tended to be higher and those with IgM lower in the diabetics than in the controls, although the differences were not statistically significant. The differences do not seem to be etiologically related since they are more obvious in older children whose diabetes must have started at least several years prior to the study.

On the other hand, B-cells were high in control children younger than six years of age as compared with the older controls. A similar age-related phenomenon has been reported in normal children by

Ugazio and associates.[26] The absence of such an age-dependent increase in diabetics might be abnormal, but the number of patients in this age group is too small to allow any conclusion.

Increased numbers of T-lymphocytes in the blood or in the tissues have been reported in a variety of autoimmune diseases; T-cells were found increased in the peripheral blood of patients with autoimmune thyroiditis,[17] in the labial salivary glands in patients with Sjögren's syndrome,[18] and in the synovial fluid of patients with rheumatoid arthritis.[19] Normal quantities of T-cells in the peripheral blood in patients with juvenile diabetes, however, do not necessarily preclude the possibility of an autoimmune sensitization of T-lymphocytes. The increased proliferative response of lymphocytes from patients with insulin-dependent diabetes on stimulation by insulin and insulin fragments[12] and recent detection of anti-islet cell antibody in these patients[6-9] strongly suggest the existence of clones of lymphocytes sensitized to pancreatic antigens which might be insufficient in number to be reflected in the total T-cell counts unless they have been caused to proliferate by the specific antigenic stimuli.

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