Monoclonal Antibodies Defined Abnormalities of T-Lymphocytes in Type I (Insulin-dependent) Diabetes

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SUMMARY

Peripheral T-lymphocyte subsets have been investigated in 36 patients with type I (insulin-dependent) diabetes of varying duration, 18 patients with type II (non-insulin-dependent) diabetes, and in 23 healthy subjects, using six different monoclonal antibodies. At the time of diagnosis of type I diabetes, there was evidence of an increase in cytotoxic T-lymphocytes, a decrease in suppressor T-lymphocytes, but a normal proportion of helper/inducer T-lymphocytes. In six of seven newly diagnosed cases studied, there was evidence of an increased number of activated T-cells. An increase in activated T-cells was also found in 5 of 10 genetically susceptible islet cell antibody positive unaffected siblings of type I diabetic probands. In type I diabetes of long standing, the total T-cell population was decreased, largely due to a marked decrease in helper/inducer T-lymphocytes. Type II diabetic patients showed no abnormalities in T-lymphocyte subsets, making it unlikely that hyperglycemia was responsible for the changes observed.

These results suggest that an imbalance of T-lymphocyte regulation is an important feature of type I diabetes and lend support for an immunologic role in its early pathogenesis. DIABETES *32*:91–94, January 1983.

The pathologic hallmark of type I (insulin-dependent) diabetes is the selective destruction of the pancreatic islet B-cells. In the early stages, the islets may be infiltrated by mononuclear cells. This phenomenon, "insulitis," has been found in a substantial proportion of newly diagnosed cases[1] and may be compatible with a recent viral infection or with a cell-mediated immune response causing B-cell destruction.

During the past few years there have been conflicting reports concerning possible abnormalities of T-lymphocytes in type I diabetes.[2–4] Evidence for possible cell-mediated cytotoxic activity in the early pathogenesis of the disease has been provided by the findings of elevated K-cell levels in both newly diagnosed cases and genetically susceptible siblings of type I diabetics.[5] Elevated K-cell levels were also shown to correlate with antibody-dependent cytotoxicity using nonspecific and specific target cells.[6–8] There are also reports of reduced suppressor cell function at the time of diagnosis.[9] It is not well established whether any of these abnormalities reflect an imbalance of T-cell subsets because of the poor specificity of the techniques previously used in the determination of lymphocyte subpopulations. Recently, advances in the understanding of the complex events taking place in the immune response have been made by the use of monoclonal antibodies.

In this study we report the existence of altered T-lymphocyte subsets in type I diabetic patients, demonstrated using monoclonal antibodies. These findings lend support to the concept that there is a cell-mediated autoimmune response involved in the destruction of islet B-cells in this disease.

SUBJECTS AND METHODS

Subjects. We studied the following subjects: (1) 16 patients with type I diabetes (10 males and 6 females, mean age 15 yr; range: 10–31 yr) within 3 days or less of diagnosis: blood was collected before the initiation of insulin treatment; (2) 20 patients with type I diabetes of long duration, ranging from

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12 to 30 yr (12 males and 8 females, mean age 46 yr; range: 28–64 yr); (3) 18 patients with type II (non-insulin-dependent) diabetes, duration of disease 11–30 yr (8 males and 10 females, mean age 56 yr; range: 40–75 yr); (4) 10 unaffected siblings of type I diabetic probands. All of these had islet cell antibodies (6 complement fixing) and 8 were either HLA identical or haploidentical with the proband; and (5) 23 healthy subjects (13 males and 10 females, mean age 28 yr; range: 22–45 yr).

Preparation of peripheral mononuclear cells. Fifteen milliliters of heparinized venous blood was fractionated by the standard lymphoprep gradient centrifugation technique.[10] Recovered cells were washed, suspended in a mixture of 90% fetal calf serum and 10% dimethyl sulfoxide, and then frozen at −70°C in cryotubes embedded in polystyrene. Cells were stored until required.

Monoclonal antibodies. The following monoclonal antibodies were used: (1) UCHT1, reacting with cell surface antigens of 90% of total T-rosette-forming cells;[11] (2) 3A1 (kindly provided by Dr. G. Eisenbarth, Joslin Clinic, Boston, Massachusetts), defining helper/inducer and suppressor T-cells;[12] (3) Leu 3A (Becton-Dickinson), defining helper/inducer T-cells;[13] (4) UCHT4, identifying cytotoxic/suppressor cells: UCHT4 (as Leu 2A and OKT8) recognize closely related antigenic determinants on cytotoxic/suppressor cells (Beverley, unpublished data); and (5) 4F2 and 5E9 (kindly provided by Dr. G. Eisenbarth), defining activated T-cells.[14,15]

Procedures. For each subject, $5.0 \times 10^5 \times$ cells/tube were incubated separately with 50 μl of the appropriate dilution of each monoclonal antibody for 30 min at 4°C. Since 4F2 monoclonal antibody binds either to activated lymphocytes or monocytes, defibrinated blood was used to avoid monocyte contamination in the final cell preparation in this case. After washing twice with Hank's balanced salt solution, the cells were incubated with fluorescein-conjugated rabbit antimouse antibody (Nordic Laboratories Ltd.) for 30 min at 4°C and washed twice more. The percentage of cells positive for rim fluorescence with each of the monoclonal antibodies was determined with a Leitz fluorescent microscope equipped for epifluorescence. For each slide, 200 cells were read. After verification of a normal distribution, the Student *t* test for unpaired data was used to determine significance between the values.

RESULTS

The results of lymphocyte typing, expressed as the percentage (mean ± SD) of cells reacting with a given monoclonal antibody are shown in Table 1. Newly diagnosed type I diabetic patients showed a significant increase in the proportion of the UCHT4 positive cell subset ($P < 0.01$) and a significant decrease of the proportion of the 3A1 positive cell subset ($P < 0.001$) compared with healthy subjects. The proportions of the UCHT1 positive cells and Leu 3A positive cells were not significantly different from the proportions found in the healthy subjects.

The patients with type I diabetes of long duration showed a significantly decreased proportion of the UCHT1 positive cells ($P < 0.001$), Leu 3A positive cells ($P < 0.001$), and 3A1 positive cells ($P < 0.001$) when compared with the healthy subjects. However, the UCHT4 positive cell subset remained significantly increased ($P < 0.05$) (Table 1).

In patients with type II diabetes of long duration, we did not find any significant difference in the proportions of Leu 3A positive or UCHT4 positive cell subpopulations compared with the healthy subjects (Table 1).

The results relating to activated T-cells as defined by the 4F2 and 5E9 monoclonal antibodies are shown in Table 2. Thus, six of seven newly diagnosed diabetics studied had evidence of T-cell activation; of the unaffected siblings, 5 of 10 had evidence of activated T-cells.

DISCUSSION

This study provides evidence that abnormalities of T-cell regulation are detectable in patients with type I diabetes. However, the abnormalities found at the time of diagnosis are different from those found in patients with disease of long standing. Thus, in newly diagnosed cases, the main alteration found was an increase in the cytotoxic/suppressor T-cell subset (UCHT4 positive) accompanied by a decrease in some cells belonging to the 3A1 subset. The 3A1 positive subset includes both helper/inducer and some suppressor T-cells, but it is likely that the decrease is related to suppressor rather than helper/inducer cells since the proportion of Leu 3A positive cells was normal and Leu 3A is thought to define specifically the helper/inducer subset. The data therefore suggest an increase in the cytotoxic subset, although any conclusions concerning the functional aspects

TABLE 1
Percentage of positive lymphocytes with different monoclonal antibodies in patients with type I diabetes of different duration, patients with type II diabetes, and normal subjects (mean ± SD)

Monoclonal antibodies	Newly diagnosed type I diabetics (N = 16)	Long-standing type I diabetics (N = 20)	Long-standing type II diabetics (N = 18)	Normal subjects (N = 23)
% UCHT1 (90% peripheral T-lymphocyte)	52 ± 9*	46 ± 6§	—	54 ± 6
% 3A1 (helper/inducer/suppressor)	34 ± 5§	35 ± 8§	—	43 ± 4
% Leu 3A (helper/inducer)	28 ± 7*	21 ± 6§	28 ± 5*	30 ± 3
% UCHT4 (cytotoxic/suppressor)	27 ± 7‡	25 ± 5†	22 ± 7*	21 ± 5

P value versus normal subjects: *NS; †$P < 0.05$; ‡$P < 0.01$; and §$P < 0.001$.

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TABLE 2
Percentage of activated T-cells as defined by monoclonal antibodies 4F2 and 5E9 in patients with newly diagnosed, type I diabetes and in genetically susceptible islet cell antibody (ICA) positive unaffected siblings of type I diabetic probands

	4F2	5E9
Mean percentage in normal subjects	2 ± 1 (N = 15)	2 ± 1 (N = 12)
Newly diagnosed type I diabetics (case nos.)		
1	15	NT†
2	10	NT
3	20	NT
4	20	NT
5	17	NT
6	13	NT
7	2	0
ICA$^+$ subjects*		
1	1	0
2	16	9
3	6	1
4	3	1
5	9	3
6	13	14
7	8	NT
8	17	13
9	10	11
10	1	1

*ICA$^+$ = islet cell antibody positive.
†NT = not tested.

of these cells' function, based only on surface phenotype, remain speculative. Nevertheless, the findings are consistent with the hypothesis that there is an imbalance of T-cell subsets in the early pathogenesis of the disease. Further support for this is provided by an increased number of activated (Ia-antigen-bearing) T-lymphocytes. These results are consistent with those reported recently by Jackson et al.[16] with the exception that their results using the 4F2 monoclonal antibody did not show an excess of activated T-lymphocytes. A similar finding has also been found in patients with Graves' disease and in patients with rheumatoid arthritis.[17] Graves' disease is strongly associated with HLA-DR3 and rheumatoid arthritis with -DR4. Ninety-eight percent of type I diabetic patients possess either -DR3 or -DR4 or both of these antigens.[18] No increase in activated T-cells was found in patients with type II diabetes. An important additional finding of the present study was the finding of increased numbers of activated T-lymphocytes in 5 of 10 islet cell antibody positive, genetically susceptible but otherwise normal, healthy subjects of type I diabetic probands. This might suggest that full expression of neoantigens on these activated cells is closely related to an early stage of B-cell destruction.

In patients with type I diabetes of long duration, T-cell subset abnormalities were still present but were of a different nature. It appears that in these patients the total T-cell population was decreased, confirming previous results,[6] and this was largely due to a decrease in helper/inducer (Leu 3A) cells with a concomitant increase of suppressor cells, which might help to explain the generally impaired immune function in these patients.[19] In addition, the increase in the proportion of cytotoxic/suppressor cells (UCHT4) may diminish adverse effects of insulin treatment (i.e., insulin antibodies, allergic reactions, delayed hypersensitivity) by the production of specific suppressor factors.[20]

Finally, it is of interest that none of these abnormalities were detected in patients with type II diabetes, suggesting that the hyperglycemia itself is not responsible for the abnormalities in lymphocyte subsets in classical type I diabetic patients. If the present findings are confirmed, it will be possible to use such techniques to monitor early abnormalities of T-cell function during the initial pathogenesis of type I diabetes, particularly in genetically susceptible siblings of type I diabetic probands.

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