Peripheral blood T-cell subsets studied by monoclonal antibodies in Type 1 (insulin-dependent) diabetes: effect of blood glucose control

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Summary. Peripheral T lymphocyte subsets were investigated, using monoclonal antibodies, in 14 patients with acute diabetes of duration less than 1 month (before insulin treatment) and after prolonged strict blood glucose control, and also in 40 healthy volunteers. At the time of diagnosis, the percentage total T cells was decreased (67.6 ± 8.4 versus $72.8 \pm 6.6\%$), but T4 'helper' cells and T8 'suppressor/cytotoxic' cells were in the normal range. The T4/T8 ratio was not significantly higher than in the control group and B-cell percentages were increased (IgS: 18.3 ± 7.1 versus $12.4 \pm 4.9\%$). The second T cell enumeration, performed after sustained normoglycaemia,

showed a normal total T cell percentage and a decrease in the T4/T8 ratio depending on a decrease of T4 cells (38.3 ± 12.8 versus 49.3 ± 13.4), without any change of T8 lymphocytes; B cells remained elevated. These results suggest that insulin deficiency/metabolic derangement was responsible for an imbalance of circulating lymphocytes and underlines the importance of metabolic control in the assessment of such immunological parameters.

Key words: Monoclonal antibody, Type 1 diabetes, T lymphocyte subsets, metabolic control.

Numerous studies support the hypothesis that autoimmunity is involved in the pathogenesis of Type I (insulin-dependent) diabetes mellitus. The presence of the anti-islet antibodies [1] and insulinitis [2] at the early phase of the disease, the characteristic association between HLA antigens and diabetes [3], and the role of environmental factors at the onset of the disease [4] suggest that a genetically determined immune reaction is involved in the B-cell lysis. Several studies concerning cell-mediated immunity have shown a lymphocytic sensitization against pancreatic antigens in recent Type 1 diabetes [5] an altered lymphocyte response to T-cell mitogens in poorly controlled diabetes [6] and a lowered suppressor cell activity at the onset of the disease [7]. Studies concerning the enumeration of circulating blood lymphocytes have been conflicting, showing a reduction of peripheral T lymphocytes [8], an excess of B lymphocytes [9], or a normal lymphocyte population [10]. These discrepancies may be explained by methodological considerations, patient selection, or degree of metabolic control, as reported previously [6]. More recently, T lymphocyte subpopulations have been investigated using monoclonal antibodies, but results still remain conflicting.

In an attempt to define these differences, we have re-evaluated the influence of metabolic control on the

phenotype of circulating blood lymphocytes and specially of T lymphocyte subsets using monoclonal antibodies.

Subjects and methods

Subjects

We studied 14 patients (seven males and seven females; median age 18 years; range 6–40 years) with acute diabetes with symptoms of less than 1 month before the initiation of insulin treatment, and 8–10 days later, after a 3-day intravenous insulin infusion to achieve sustained normoglycaemia; the insulin infusion was performed using an open loop system in which insulin infusion rates were adjusted by repeated capillary blood glucose determinations (10 per day). The control group included 40 sex- and age-matched healthy volunteers.

Methods

Heparinized venous blood (20 ml) was drawn up from each patient at 08.00 h for isolation of peripheral mononuclear cells by centrifugation on Ficoll-Hypaque gradient [11]. Monoclonal antibodies used were specifically directed against T4 'helper' cells (Leu-3a, Becton Dickinson), against T8 'suppressor/cytotoxic' cells (BL15, kindly provided by J. Brochier, Lyon, France) and against HLA-D/DR-bearing cells (BL2, J. Brochier); the detection of positive cells by each monoclonal antibody was achieved by a immunofluorescence method using fluoresceinated class-specific goat anti-mice immunoglobulins [12]. Total T cells were enumerated using classical E-rosettes and Ig-bearing B-

Table 1. Lymphocyte membrane markers in patients with acute onset of diabetes before and after continuous intravenous insulin infusion compared with control subjects

Lymphocyte membrane markers (%)	Control subjects (n = 40)	Type I diabetic patients (n = 14)	
		Before infusion	After infusion
Total T cells (E rosettes)	72.8 ± 6.6	67.6 ± 8.4	68.9 ± 9.6
T4 'helper' cells (Leu3a)	49.3 ± 13.4	47.4 ± 14.2	38.3 ± 12.8
T8 'suppressor/cytotoxic' cells (BL15)	30.5 ± 10.6	26.1 ± 10.8	24.9 ± 10.4
T4/T8 ratio (Leu3a/BL15)	1.7 ± 0.5	2.1 ± 1.0	1.8 ± 0.6
B-cells (IgS)	12.4 ± 4.9	18.3 ± 7.1	17.3 ± 7.8
HLA-D/DR-positive cells (BL2)	14.8 ± 3.9	19.0 ± 5.7	17.4 ± 11.0
Monocytes (peroxidase)	15.6 ± 7.8	18.5 ± 10.1	19.6 ± 11.3

Results expressed as mean ± SD

cells were detected by membrane immunofluorescence [6]. Finally, monocyte contamination was assessed using peroxidase staining.

Results of lymphocyte enumeration were expressed as the percentage (mean ± SD) and statistical analysis was carried out using Student's t-test.

Results (Table 1)

The first enumeration of T cells performed at diagnosis before insulin therapy (blood glucose: 17.1 ± 2.8 mmol/l) showed a significantly decreased percentage of total T cells in peripheral blood compared with the control subjects ($p < 0.05$) but Leu3a (T4) and BL15 (T8) positive cells were in the normal range. The T4/T8 ratio was not significantly increased in the diabetic patients (2.1 ± 1.0 versus 1.7 ± 0.5). Conversely, the B-cell percentage evaluated by immunofluorescence was significantly increased ($p < 0.01$) as were the BL2-positive cells ($p < 0.01$) compared with the control group. After sustained normoglycaemia (blood glucose: 5.5 ± 0.6 mmol/l), the second T-cell enumeration revealed that total T-cell percentages increased and were similar to the controls. At the same time, the T4/T8 ratio decreased to the normal range (1.8 ± 0.6) due to a lower percentage of Leu3a positive cells. After good metabolic control, Ig-bearing B cells remained elevated ($p < 0.02$), but BL2-positive cells were similar to controls. Proportions of peroxidase-positive cells were within the normal range before and after strict metabolic control.

Discussion

Abnormalities of circulating blood lymphocytes were detectable in recently diagnosed diabetes of acute onset before initiation of insulin treatment. In agreement with other authors, we found a reduction of the percentage of total T cells (8,13). This T-cell defect did not appear to be due to an abnormal regulation of T cell subsets as reported elsewhere [14], because T4 'helper' and T8 'suppressor/cytotoxic' cells remained in the normal range. Nevertheless, as in other reports [8, 15, 16], our T4/T8 ratio was elevated, but this increase did not reach statistical significance. B-cells proportions, as assessed by Ig-bearing cell enumeration, were elevated in diabetic patients of recent onset; this finding has been reported in islet-cell antibody-positive diabetes [9] and may reflect the extent of autoantibody production. BL2-positive cells, recognized by a monomorphic anti-HLA-D/DR monoclonal antibody, increased concurrently; this result is in disagreement with an elevated percentage of activated T cells in recently diagnosed diabetes reported previously [17]. After optimal blood glucose control, the number of total T cells was normalized; the total T-cell defect appeared reversible and closely dependent on metabolic status. During normoglycaemia, the T4/T8 ratio dropped to the normal range, due to a significant decrease of T4 cells without any change of T8 lymphocytes. Complete normalization of the immunoregulatory ratio (T4/T8 ratio) emphasizes the influence of metabolic abnormalities upon T cell subset enumeration. Excess of Ig-bearing cells was not corrected completely by adequate metabolic control, but BL2-positive cell percentage decreased to normal.

In conclusion, this study underlines the importance of metabolic control in the assessment of immunological parameters, such as circulating lymphocytes, and in particular T-cell subsets. Even if defects of cellular immunity appear to be secondary to abnormal glucose metabolism, they may favour pancreatic attack by environmental factors, such as viral infections.

References

1. Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2: 1279-1283
2. Gepts W, Lecompte PhM (1981) The pancreatic islets in diabetes. *Am J Med* 70: 105-115
3. Farid NR, Bear JC (1981) The human major histocompatibility complex and endocrine disease. *Endocr Rev* 2: 50-86
4. Tattersall R, Pyke DA (1972) Diabetes in identical twins. *Lancet* 2: 1120-1125
5. MacCuish AC, Jordan J, Campbell CJ, Duncan LJP, Irvine WJ (1974) Cell-mediated immunity to human pancreas in diabetes mellitus. *Diabetes* 23: 693-697
6. Selam JL, Clot J, Andary M, Mirouze J (1979) Circulating lymphocyte subpopulations in juvenile insulin-dependent diabetes. *Diabetologia* 16: 35-40
7. Buschard K, Madsbad S, Rygaard J (1980) Depressed suppressor

- cell activity in patients with newly diagnosed insulin-dependent diabetes mellitus. *Clin Exp Immunol* 41: 25-32
8. Mascart-Lemone F, Delespesse G, Dorchy H, Lemiére B, Servais G (1982) Characterization of immunoregulatory T lymphocytes in insulin-dependent diabetic children by means of monoclonal antibodies. *Clin Exp Immunol* 47: 296-300
 9. Bersani G, Zanco P, Padovan D, Betterle C (1981) Lymphocyte subpopulations in insulin-dependent diabetics with and without serum islet-cell autoantibodies. *Diabetologia* 20: 47-50
 10. MacCuish AC, Urbaniak SJ, Campbell CJ, Duncan LJP, Irvine WJ (1974) Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetes. *Diabetes* 23: 708-712
 11. Boyum A (1968) Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 21: 31-37
 12. Reinherz EL, Kung PC, Goldstein G, Schlossman SF (1979) Separation of functional subsets of human T cells by monoclonal antibody. *Proc Natl Acad Sci USA* 76: 4062-4065
 13. Quiniou MC, Debray-Sachs M, Assan R (1983) Modifications des sous-populations des lymphocytes T et immunité anti-îlots chez les diabétiques insulino-dépendants. *Diabet Metab* 9: 173-174 (Abstract)
 14. Pozzilli P, Zuccarini O, Iavicoli M, Andreani D, Sensi M, Spencer KM, Bottazzo GF, Beverley PCL, Kyner JL, Cudworth AG (1983) Monoclonal antibodies defined abnormalities of T-lymphocytes in Type I (insulin-dependent) diabetes. *Diabetes* 32: 91-94
 15. Buschard K, Röpke C, Madsbad S, Mehlsen J, Sørensen TB, Rygaard J (1982) Alterations of peripheral T-lymphocyte subpopulations in patients with insulin-dependent (Type I) diabetes mellitus. In: Alberti KGMM, Ogada T, Aluoch TA, Mngola EN (eds) *Proc 11th Congress of the International Diabetes Federation*, Nairobi, Kenya, Excerpta Medica, Amsterdam, p 42 (Abstract)
 16. Galluzzo A, Giordano C, Sparacino G, Camarda G, Rubino G, Bompiani GD (1982) Suppressor cell deficit using monoclonal antibodies in recent-onset Type I diabetes mellitus: evidence of statistically independent models in lymphocyte subsets. In: Albert KGMM, Ogada T, Aluoch JA, Mngola EN (eds) *Proc 11th Congress of the International Diabetes Federation*, Nairobi, Kenya Excerpta Medica, Amsterdam p 43 (Abstract)
 17. Jackson RA, Morris MA, Haynes BF, Eisenbarth GS (1982) Increased circulating Ia-antigen-bearing T cells in Type I diabetes mellitus. *New Engl J Med* 306: 785-788

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