Clin. exp. Immunol. (1982) **47**, 296–300.

Characterization of immunoregulatory T lymphocytes in insulin-dependent diabetic children by means of monoclonal antibodies

F. MASCART-LEMONE,* G. DELESPESSE,* H. DORCHY,† B. LEMIERE† & G. SERVAIS* **Department of Immunology*, †*Department of Pediatrics, Saint-Pierre Hospital, Brussels Free University, Brussels, Belgium*

(Accepted for publication 21 August 1981)

SUMMARY

Monoclonal antibodies of the OKT series were used to identify total T lymphocytes ($OKT3^+$) and their helper ($OKT4^+$) and suppressor-cytotoxic ($OKT8^+$) subsets in 34 insulin-dependent diabetic children and in 39 young healthy controls. The diabetics have a moderate but significant reduction of the proportions of total T lymphocytes at the expense of the T helper subset; the proportions of suppressor-cytotoxic cells are normal. These abnormalities of the lymphocyte subpopulations are not related to the duration of diabetes, the control of the disease nor to the presence of autoimmune phenomena. The latter were found in 51·4% of the patients.

INTRODUCTION

Numerous investigations have been performed, aiming at a better understanding of the high susceptibility of diabetic patients to infections of bacterial, viral or mycotic origins (Younger, 1965; Eisert, 1965; Thornton, 1971). Staphylococcal infections, which are particularly frequent in poorly controlled diabetes, have been related to partial defects of the polymorphonuclear leucocytes rather than to a defective antibody production (Perillie, Nolan and Finch, 1962; Bybee and Rogers, 1964; Mowat and Baum, 1971; Bagdade, Root and Bulger, 1974). The susceptibility of insulin-dependent diabetics (IDD) to mycotic and tuberculous infections led to the suspicion of an alteration of their cell mediated immunity. Most studies in the *in vitro* lymphocyte response to T cell-mitogens showed a depressed response, particularly in poorly controlled cases (Delespesse *et al.*, 1974; Selam *et al.*, 1979; MacCuish *et al.*, 1974); however Stratton *et al.* (1977) found that diabetics have a normal response to phytohemagglutinin (PHA). Similar discrepancies are found when comparing reports on the peripheral blood lymphocyte subpopulations. These differences may be explained not only by methodological considerations but also by the ways in which patients are selected and in the appreciation of their degree of metabolic control. Using the rosette formation test to identify T lymphocytes, some authors found a decrease in the absolute numbers of peripheral blood T lymphocytes in IDD (Cattaneo, Saibene & Pozza, 1976; Müller *et al.*, 1980); others found this defect only in poorly controlled patients (Selam *et al.*, 1979), whereas several studies showed normal numbers of circulating T lymphocytes in IDD (MacCuish *et al.*, 1974; Hann, Kaye & Falkner, 1976; Stratton *et al.*, 1977). Less data are available on the T lymphocyte subpopulations in these patients.

Correspondence: Dr G. Delespesse, Département d'Immunologie, Hôpital Universitaire Saint-Pierre, rue Haute 322, 1000 Brussels, Belgium.

0009-9104/82/0200-0296$02.00

Müller *et al.* (1980) found that the moderate reduction in the numbers of peripheral T lymphocytes in IDD is secondary to a decrease of cells forming early or 'active' rosettes. Selam *et al.* (1979) described a reduction of T lymphocytes bearing membrane receptors for the Fc portion of IgG (Tγ) in all diabetics, irrespective of the metabolic control of the disease. Using a functional assay, based on the activation of suppressor-cells by Concanavalin A, Buschard, Madsbad & Rygaard (1980) found a reduced suppressor cell activity in IDD of recent onset. These authors suggested that the defective suppressor-cell's activity should be related to the presence of autoantibodies to pancreatic islet cells. On the other hand, Plouffe *et al.* (1978) recently reported that two uncontrolled IDD showed an excessive suppressor activity of adherent cells on the patient lymphocyte response to PHA. The recent availability of well characterized monoclonal antibodies specific for total T lymphocytes and their helper and suppressor-cytotoxic subsets allows a more precise evaluation of the human immunoregulatory T cells (Kung *et al.*, 1979; Reinherz & Schlossman, 1980; Reinherz *et al.*, 1979). In the present study we have investigated, by means of monoclonal antibodies of the OKT series, the T lymphocyte subpopulations in 34 insulin-dependent diabetic children compared to 39 healthy young adults. The findings have been analysed in relation to the duration of the disease, the metabolic control, the presence of retinopathy and the existence of tissue auto-antibodies.

MATERIALS AND METHODS

Participants. 34 insulin-dependent diabetic children (15 boys and 19 girls) aged from 5 to 21 years have been tested. The duration of the disease ranged from 2 months to 15 years. Metabolic control was estimated by measuring the day of the test, A_{1c} glycosylated haemoglobin, using an electrofocusing method (Schoos, Schoos-Barbette & Lambotte, 1978). A_{1c} glycosylated haemoglobin level depends on the mean glucose concentration to which the red cells have been exposed during their lifespan; in normal individuals less than 6% of haemoglobin is glycosylated. In our study, 18 children (53%) had more than 7% of glycosylated haemoglobin. Six patients had retinal vascular changes as objectivated by fluorescein angiography (Dorchy *et al.*, 1977). Mean insulin requirements (± 1 s.d.) were $1{\cdot}0 \pm 0{\cdot}4$ units/kg body weight. None of the participants had clinical evidence of nephropathy. The control subjects were technicians or blood donors of less than 40 years old.

Lymphocyte surface markers. Monoclonal antibodies were obtained from Ortho Pharmaceutical Corporation (Raritan, New Jersey, USA). The antibodies used in this study were directed against mature thymocytes and all peripheral blood T cells (OKT3), T helper cells (OKT4), T suppressor-cytotoxic cells (OKT8) and Ia antigen (OKI1). The specificity of these antibodies has been well documented (Kung *et al.*, 1979; Reinherz *et al.*, 1979; Reinherz *et al.*, 1980). Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation of heparin-treated venous blood.

These cells were first incubated for 30 min with the monoclonal antibody and then labelled with a fluorescein-conjugated goat anti-mouse serum (GAM-FITC, Tago-Lab., Netherlands). Fluorescent cells were counted by means of a Leitz fluorescent microscope; counting was performed by a technician ignoring the origin of the sample and the nature of the monoclonal antibody. A negative control (cells treated only with GAM-FITC) was included in each preparation of peripheral blood mononuclear cells; the value of this control was subtracted for the calculation of the results. These are expressed as percentages of all cells in the preparation. Student's *t*-test was used for the statistical evaluation.

Detection of autoantibodies. Each serum was tested by a routine indirect immunofluorescence technique for the presence of antibodies against 11 different antigens. Substrates consisted of cryostat sections of the following freshly frozen organs; kidney, liver, heart and abdominal muscles from rat origin; ovary from monkey and skin from rabbit origin; stomach, thyroid, sub-maxillary gland, adrenal and pancreas blood group O of human origin. The tagged antibody was a polyvalent anti-human Ig of swine origin (swine anti-human Ig FITC batch No. 277 Nordic Immunological Labs., The Netherlands).

Table 1. Lymphocyte subpopulations in insulin-dependent diabetic children

	OKT3 (%)	OKT4 (%)	OKT8 (%)	OKT4/OKT8	OKI1 (%)
Controls (39)	72·7±8·5*	45·9±9·7	24·1±6·8	2·2±1·5	10·5±4·5
Patients (34)	68·5±8·6	39·5±9·4	24·8±5·3	1·6±0·5	13·8±6·3
Significance	$P<0·05$	$P<0·005$	ns	$P<0·025$	$P<0·020$

* Results are expressed as the mean ± the standard deviation.

Table 2. T lymphocyte subpopulations in diabetic children

	n	OKT3 (%)	OKT4 (%)	OKT8 (%)	OKT4/OKT8	OKI1 (%)
Sex: male	15	66·2±9·4*	36·6±8·7	23·6±6·2	1·62±0·64	13·3±5·7
female	19	70·3±7·5	41·8±9·2	25·8±4·2	1·63±0·40	13·8±6·6
Onset of the disease: < 1 yr	10	70·5±8·2	40·6±8·4	26·0±6·0	1·60±0·38	14·6±5·6
> 1 yr	23	69·0±5·7	39·8±8·7	24·4±5·1	1·68±0·54	13·4±6·5
Well controlled diabetics—(Hb $A_{1c}<6$)	8	72·5±6·2	42·3±8·5	25·0±6·8	1·7±0·5	13·2±5·7
Fairly or poorly controlled diabetics—(Hb $A_{1c}>7$)	18	67·1±10·1	39·5±10·8	25·3±4·6	1·6±0·5	14·1±5·8
Patients without autoantibodies	16	67·9±10·3	40·6±10·5	24·2±5·4	1·70±0·58	12·3±5·5
Patients with autoantibodies	17	68·5±6·8	37·9±8·0	25·1±5·2	1·54±0·46	15·2±6·7
Patients without retinopathy	19	68·7±6·7	40·8±8·8	24·0±4·2	1·71±0·5	12·9±6·8
Patients with retinopathy	6	71·0±4·8	38·0±9·4	27·0±5·8	1·46±0·6	14·8±7·2

* Results are expressed as the mean ± s.d.

RESULTS

Lymphocytes studies (Table 1)

Diabetics had a moderate but significant reduction of the proportions of $OKT3^+$ cells when compared to the controls ($P<0·05$). Analysis of the subpopulations of T lymphocytes indicated that this decrease in total T lymphocytes was secondary to a reduction of the T helper subsets ($OKT4^+$; $P<0·005$); the percentages of suppressor-cytotoxic T cells were comparable in the two groups of participants. The ratio between T helper/T suppressor cells was significantly lower in the diabetics ($P<0·025$). The proportion of cells bearing Ia antigen was significantly higher in the diabetics ($P<0·05$).

Correlations between lymphocyte alterations and various clinical parameters (Table 2)

There was no relationship between the results of the lymphocyte studies and any of the following clinical parameters; sex, duration of the disease, level of A_{1c} glycosylated haemoglobin, presence of autoantibodies and existence of retinal vascular changes.

DISCUSSION

In the present study of 34 insulin-dependent diabetic children we observed a reduction in the percentages of circulating T helper cells concomitant with a decrease in the proportions of total T lymphocytes. These alterations of the peripheral blood T lymphocytes are moderate and cannot

explain some previous observations of a striking impairment in these patients of peripheral blood lymphocytes response to PHA. This view is further supported by the finding of comparable proportions of $OKT3^+$ and $OKT4^+$ lymphocytes in well controlled and in poorly controlled subjects, whereas it has been reported that the latter have a much more pronounced alteration of their *in vitro* lymphocyte response to T cell mitogens (Delespesse *et al.*, 1974; MacCuish *et al.*, 1974; Selam *et al.*, 1979).

Two observations argue against the concept that the high incidence of autoimmune phenomena in diabetes mellitus could be secondary to an alteration of their suppressor cells. First, the proportions of T lymphocytes with the cytotoxic-suppressor phenotype were normal in the diabetics. Second, the proportions of immunoregulatory T cells were comparable in patients with and without antibodies. Note that the former comprised 51·5% of the participants, a figure which is comparable to our previous findings in children (Dorchy *et al.*, 1981) and in adult IDD (Delespesse *et al.*, 1980). As already mentioned, previous workers have shown a reduction of T suppressor cell numbers or function in diabetic patients. Selam *et al.* (1979) found that diabetic children had a decrease of circulating $T\gamma$ cells, which were generally considered to be the T suppressor-cells. The present data are not in opposition with this study since it has been clearly demonstrated that $OKT8^+$ and $T\gamma$ cells are not identical populations. Reinherz *et al.* (1980) have indeed shown that highly purified preparations of $OKT8^+$ cells contain the same proportions of $T\gamma$ cells as unfractionated T lymphocytes or purified $OKT4^+$ cells; conversely purified $T\gamma$ cells are not enriched in $OKT8^+$ cells. Comparison of the present results to those of Buschard *et al.* (1980) is more difficult since they used a functional assay to assess the activity of suppressor cells in diabetic patients. *A priori*, the presence of a normal number of $OKT8^+$ cells in a given lymphocyte preparation is not incompatible with the findings, in this study, of a striking defect in the Con A induced suppressor activity. Indeed, the suppressor activity is dependent not only upon $OKT8^+$ cells but also upon interactions between these cells and subsets of $OKT4^+$ lymphocytes, as well as monocytes (Reinherz & Schlossman, 1981).

We have no explanation for the high proportions of cells bearing Ia antigen in the diabetics; these cells could be either B cells, monocytes or even activated T cells (Reinherz *et al.*, 1979).

In conclusion, we suggest that the minor alterations in the proportions of T lymphocytes and their subsets do not account for the high incidence of autoimmune reactions nor for the suspected defective cell-mediated immunity in patients with insulin dependent diabetes.

The authors thank Professor D. Toussaint for performing the fluorescein angiographies.

REFERENCES

BAGDADE, J.D., ROOT, R.K. & BULGER, R.J. (1974) Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes*, **23**, 9.

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.

BYBEE, J.D. & ROGERS, D.E. (1964) The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus. *J. Lab. clin. Med.* **64**, 1.

CATTANEO, R., SAIBENE, V. & POZZA, G. (1976) Peripheral T-lymphocytes in juvenile-onset diabetics and in maternity-onset diabetics. *Diabetes*, **25**, 223.

DELESPESSE, G., DUCHATEAU, J., BASTENIE, P.A., LAUVAUX, J.P., COLLET, H. & GOVAERTS, A. (1974) Cell-mediated immunity in diabetes mellitus. *Clin. exp. Immunol.* **18**, 461.

DELESPESSE, G., GAUSSET, P., SARFATI, M., DUBI-RUCQUOY, M., DEBISSCHOP, M.J. & VAN HAELST, L. (1980) Circulating immune complexes in old people and in diabetics: correlation with autoantibodies. *Clin. exp. Immunol.* **40**, 96.

DORCHY, H., TOUSSAINT, D., DEVROEDE, M., ERNOULD, C. & LOEB, H. (1977) Diagnostic de le rétinopathie diabétique infantile par angiographie fluorescéinique. Description des lésions initiales. *Nouv. Presse Med.* **6**, 345.

DORCHY, H., LEMIERE, B., TOUSSAINT, D. & GAUSSET, PH. (1981) Anticorps anti-cellules des ilôts de Langerhans et spécifiques d'organes chez les enfants et les adolescents diabétiques. Relation avec l'âge, le début et la durée du diabète, la sécrétion résiduelle d'insuline et la rétinopathie. *Nouv. Presse Med.* **10**, 2795.

EISERT, J. (1965) Diabetes and diseases of the skin. *Med. clin. North Am.* **49**, 621.

HANN, S., KAYE, R. & FALKNER, B. (1976) Subpopulations of peripheral lymphocytes in juvenile diabetes. *Diabetes*, **25**, 101.

KUNG, P.C., GOLDSTEIN, G., REINHERZ, E.L. &

SCHLOSSMAN, S.F. (1979) Monoclonal antibodies defining distinctive human T cell surface antigens. *Science*, **206**, 347.

MACCUISH, A.C., URBIANAK, S.J., CAMPBELL, C.J., DUNCAN, L.J.P. & IRVINE, W.J. (1974) Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetes. *Diabetes*, **23**, 708.

MOWAT, A.G. & BAUM, J. (1971) Chemotaxis of polymophonuclear leukocytes from patients with diabetes mellitus. *New Engl. J. Med.* **284**, 621.

MÜLLER, R., KOLB, H., KUSCHAK, D., JÖRGENS, V. & GRIES, F.A. (1980) Analyses of T-lymphocyte subpopulations in juvenile-onset diabetics. *Clin. exp. Immunol.* **39**, 130.

PERILLIE, P.E., NOLAN, J.P. & FINCH, S.C. (1962) Studies of the resistance to infection in diabetes mellitus: local exudative cellular response. *J. Lab. clin. Med.* **59**, 1008.

PLOUFFE, J.F., SILVA, J., FEKETY, R. & ALLEN, J.L. (1978) Cell-mediated immunity in diabetes mellitus. *Infect. Immun.* **21**, 425.

REINHERZ, E.L., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1979) Further characterization of the human inducer T cell subset defined by monoclonal antibody. *J. Immunol.* **123**, 2894.

REINHERZ, E.L., MORETTA, L., ROPER, M., BREARD, J.M., MINGARI, M.C., COOPER, M.D. & SCHLOSSMAN, S.F. (1980) Human T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies: a comparison. *J. exp. Med.* **151**, 969.

REINHERZ, E.L. & SCHLOSSMAN, S.F. (1980) Regulation of the immune response inducer and suppressor T-lymphocyte subsets in human beings. *New Engl. J. Med.* **303**, 370.

REINHERZ, E.L. & SCHLOSSMAN, S.F. (1981) The characterization of function of human immunoregulatory T lymphocyte subsets. *Immunol. Today*, **2**, 69.

SCHOOS, R., SCHOOS-BARBETTE, S. & LAMBOTTE, C. (1978) Dosage of hemoglobin A_{1c} by isoelectrofocusing. *Clin. Chem. Acta*, **86**, 61.

SELAM, J.L., CLOT, J., ANDARY, M. & MIROUZE, J. (1979) Circulating lymphocyte subpopulations in juvenile insulin-dependent diabetes. *Diabetologia*, **16**, 35.

STRATTON, J.A., NIES, K.M., LOUIE, J.S. & SPERLING, M.A. (1977) Cellular immunity in juvenile diabetes mellitus. *Diabetes*, **26**, 419.

THORNTON, G.F. (1971) Infections and diabetes. *Med. clin. North Am.* **55**, 931.

YOUNGER, D. (1965) Infections in diabetes. *Med. clin. North Am.* **49**, 1005.