Brief Notes and Comments

Peripheral T-Lymphocytes in Juvenile-onset Diabetics (JOD) and in Maternity-onset Diabetics (MOD)

R. Cattaneo, M.D., V. Saibene, M.D., and G. Pozza, M.D., Milan, Italy

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/3/223/348819/25-3-223.pdf by guest on 14 June 2024

SUMMARY

The percentage and absolute number per mm.^{3} of peripheral T-lymphocytes were determined in 11 juvenile-onset diabetics (JOD), in 21 maturity-onset diabetics (MOD), and in 18 normal subjects (NS). The percentage was significantly lower in JOD (38.1) than in MOD (57.2) and NS (56.5). The absolute T-lymphocyte number per mm.^{3} was significantly lower in JOD (833) than in NS (1,260); this was also true for JOD as against MOD (1,026), even if the difference was not statistically significant. No difference was found between MOD and NS, or between MOD on oral therapy and on insulin treatment. The decrease of peripheral T-lymphocytes in JOD was not related to associated illness or drugs. The data presented suggest the possibility of an altered cell-mediated immunity in juvenile-onset diabetics. DIABETES *25*:223-26, March, 1976.

Recent clinical and experimental findings suggest that autoimmune factors may have a role in the pathogenesis of diabetes mellitus, in particular of the juvenile-onset type (JOD). The histologic pattern of insulitis, detected by Gepts[1] in most JOD of recent onset, resembles the end-organ changes observed in autoimmune endocrinopathies.

The incidence of JOD in patients with overt autoimmune diseases, such as pernicious anemia, Hashimoto's thyroiditis, primary myxedema, thyrotoxicosis, idiopathic Addison's disease, hypoparathyroidism, and myasthenia gravis has been shown to be significantly increased.[2-4] These clinical observations are supported by serologic evidence of a high incidence of thyroid and gastric autoantibodies in JOD, even in the absence of clinical autoimmune disease.[2,4-6]

More direct evidence for the autoimmune hypothesis comes from the demonstration in JOD of cellular and humoral immunity to specific pancreatic antigens.

Nerup et al.[7] have shown that in about half the diabetic population they examined, almost all of the juvenile-onset type, a significant reduction of the leukocyte migration index after exposure to pancreatic antigen was present. Islet-cell autoantibodies were initially detected only in JOD with multiple immunopathies,[8,9] but more recently Maclaren et al.,[10] using human insulinoma cells as antigens, have found such autoantibodies in 34 out of 39 JOD patients. Further support for the autoimmune hypothesis comes from the detection of a high incidence of HL-A 8 histocompatibility antigen in JOD.[11,12]

The same antigen is also found significantly associated with autoimmune endocrinopathies, such as Graves' disease and idiopathic Addison's disease.[11]

This finding provides a link between genetic predisposition and autoimmunity, according to the known close association between genes controlling histocompatibility antigens and the so-called Ir genes,[13] controlling the development of cell-mediated immunity to certain antigens.

Despite extensive study on a possible autoimmune pathogenesis of diabetes mellitus, cellular mediated immunity (CMI) has not been well elucidated in JOD and in maturity-onset diabetes mellitus (MOD).

In this report, data are presented concerning the absolute number and the percentage of E-rosette-forming cells (T-lymphocytes) in the peripheral

From the Cattedra di Patologia Speciale Medica, Università degli Studi di Milano, Ospedale San Raffaele, 20090, Milano-Segrate, Italy.

Accepted for publication November 24, 1975.

blood of JOD, MOD, and normal control subjects (NS).

PATIENTS AND METHODS

Thirty-two diabetic patients were studied—11 JOD and 21 MOD. Patients were considered JOD according to their age at onset of diabetes (mean age 19.8 years) and to insulin dependence. Among MOD patients (mean age at onset 50.8 years) 13 were on oral therapy (MODO) and eight on insulin (MODI).

The patients were admitted to the hospital for metabolic control or for associated pathology (table 1); metabolic decompensation on admission was present in all JOD and in a minority of MOD (patients 13, 16, 22-25, 28, 30). Patients with diseases or those receiving drugs known to affect CMI were excluded. Poor metabolic control was determined according to the following criteria: fasting blood glucose exceeding 200 mg./100 ml. and the presence of severe glycosuria and/or ketonuria for at least one of the three days preceding examination.

Anamnestic, clinical, and laboratory details of JOD and MOD are reported in table 1.

The control group was formed of 18 normal subjects (NS)—healthy hospital employees or students—aged from 17 to 65 (mean age 34.5)—10 males and eight females. They had normal OGTT and their fasting blood glucose was in the normal range at the moment of the test; none of them had a family history of diabetes.

Counting of T-lymphocytes. The capacity of some lymphocytes to form rosettes with sheep red blood cells (E-rosettes) was used as a marker for T-cells. Lymphocytes were isolated from peripheral blood of patients and normal subjects by centrifugation on Ficoll-Hypaque, and an E-rosette test was performed according to Jondal et al.[14]

A lymphocyte surrounded by three or more SRBC was regarded a rosette (T-cell).

Rosettes were counted on a hemacytometer, and the T-cell percentage was calculated by examination of 300 lymphocytes in two different samples.

The absolute number of peripheral blood T-lymphocytes was determined on two total lymphocyte counts in the blood sample studied, according to the formula: white cells per mm.^{3} × % lymphocytes × % T-cells. The total of white cells was determined with an electronic apparatus (Haemacount). Total lymphocytes were determined by differential count, after application of May-Grünwald stain, on 200 cells.

RESULTS AND DISCUSSION

As shown in table 2, the percentage of T-lymphocytes in peripheral blood of JOD was 38.1, while in NS and in MOD it was 56.5 and 57.2, respectively. The percentage found in JOD was significantly lower ($p < 0.005$ on Student's t-test) when compared with the percentages of the other two groups. The absolute number of T-cells per mm.^{3} in peripheral blood was significantly lower in JOD than in NS (853 versus 1,261; $p < 0.05$); this value was also lower than in MOD (853 versus 1,026) even if not significantly. There was no difference, either in percentage or in absolute number, between MOD receiving oral drugs and those on insulin treatment (table 1) or between MOD and NS. The lower percentage of T-lymphocytes in JOD than in MOD appears even more relevant if the different mean age of the two groups (26.5 versus 59.7) is considered. In fact, the T-lymphocyte percentage is reported to decrease normally with aging.[15]

Because of the criteria followed in selection of patients, it seems possible to exclude any difference due to associated illness or drugs: however, the possibility that a poor metabolic control may reduce T-lymphocyte number or function must be considered. In this connection, MacCuish et al.[16] have shown a reduced transformation response to PHA in poorly controlled diabetics and have related this finding to the metabolic situation. As for rosette-forming ability, Jondal et al.[14] pointed out that this is an energy-dependent process, suppressed by metabolic inhibitors: metabolic decompensation could therefore be relevant in the interpretation of the lower values of peripheral T-lymphocytes found in diabetic patients. However, this seems not to be the case: in fact a low T-lymphocyte percentage is present only in JOD and not in MOD, and, furthermore, the T-lymphocyte percentage in five poorly controlled JOD (patients 5, 7, 8, 10, 11) was slightly higher than in six well-controlled JOD (40.4 versus 36.1) (patients 1-4, 6, 9).

Our results seem to differ from those of MacCuish et al.,[16] who did not find any difference in T-lymphocyte percentage between insulin-dependent diabetics (IDD) and normal controls.

The two investigations, however, are not comparable, because of the different criteria followed in the selection of patients.

MacCuish's report[16] is concerned with insulin-dependent diabetics (IDD), regardless of type and age of onset of diabetes, while our data suggest a signifi-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/3/223/348819/25-3-223.pdf by guest on 14 June 2024

TABLE 1

Anamnestic and clinical data, T-lymphocyte percentage, and T-lymphocytes per mm.^{3} in peripheral blood of 11 juvenile-onset diabetics (JOD), of eight maturity-onset diabetics on insulin therapy (MODI), and of 13 maturity-onset diabetics on oral therapy (MODO)

	Patient	Age/sex	Fam.	Age at onset	*Metabolic control	†Fasting blood glucose	Therapy	Associated pathology	T-cells %	T-cells/mm.^{3}
	1	12/F	+	11	G	137	In.	—	57	998
	2	15/F	+	10	G	128	In.	—	30	1,148
	3	16/F	−	16	G	84	In.	—	19	495
	4	20/M	−	20	G	165	In.	—	42	945
JOD	5	26/M	−	21	P	390	In.	—	16	426
	6	26/F	−	26	G	125	In.	—	29	1,621
	7	28/M	+	23	P	210	In.	—	65	1,759
	8	32/F	+	16	P	378	In.	Leber's s., diab. insip.	26	275
	9	34/M	−	26	G	140	In.	—	40	252
	10	39/F	+	24	P	310	In.	Leber's s., diab. insip.	37	558
	11	44/M	−	24	P	380	In.	Hypertension, diabetic retinopathy	58	682
	mean (S.E.M.)	26.5		19.8					38.1 (4.90)	833 (155.08)
	12	50/M	−	43	G	95	In.	Cerebrovascular dis.	50	1,612
	13	55/F	−	45	G	86	In.	—	56	862
	14	59/M	−	38	G	64	In.	Chronic renal fail.	66	1,441
MODI	15	61/F	−	46	P	204	In.	Hyperlipopr. II B	50	1,071
	16	62/M	−	61	P	217	In.	—	41	1,119
	17	68/F	+	64	G	170	In.	Hyperlipopr. IV	68	1,224
	18	78/F	−	66	G	131	In.	Chronic renal fail.	62	744
	19	84/F	−	70	G	125	In.	Cholangitis	66	554
	mean (S.E.M.)	64.6		54.1					57.4 (3.44)	1,078 (124.85)
	20	32/M	+	32	G	170	Or.	Hyperlipopr. II A	65	1,470
	21	32/M	+	31	G	110	Or.	—	46	309
	22	35/M	−	35	G	114	Or.	—	50	950
	23	37/M	+	37	G	168	Or.	—	53	1,240
	24	38/M	−	38	G	150	Or.	—	55	458
MODO	25	56/F	−	50	G	140	Or.	Cerebrovascular dis.	67	905
	26	61/F	+	55	G	145	Or.	Hyperlipopr. IV	59	1,532
	27	69/M	−	69	G	90	Or.	—	26	467
	28	72/M	−	42	G	145	Or.	Ischemic heart dis.	60	1,663
	29	73/F	+	60	G	94	Or.	—	48	706
	30	77/F	−	62	G	90	Or.	Chronic heart fail.	75	1,008
	31	77/F	−	57	G	160	Or.	Hypertension	73	1,402
	32	78/F	−	66	G	103	Or.	Chronic renal fail.	66	820
	mean (S.E.M.)	56.6		48.7					57.1 (3.64)	995 (122.7)

M = male; F = female; Fam. = family history of diabetes; P = poor control; G = good control; In. = insulin; Or. = oral drugs

*Assessed by evaluation of fasting blood glucose, glycosuria, and ketonuria in the three days preceding examination, according to the criteria outlined in the section Patients and Methods.

†Determined at the same time as sampling for lymphocytes study.

cant difference, as far as T-lymphocyte percentage in peripheral blood is concerned, between JOD and MOD, whether on insulin or on oral therapy.

The impairment of cell-mediated immunity in JOD, demonstrated by the data presented, deserves further investigation in order to define a possible autoimmune pathogenesis in the development of diabetes mellitus.

ACKNOWLEDGMENT

We wish to express our appreciation to Mr. Vittorio Caimi and Mr. Alberto Margonato for their skillful technical assistance.

REFERENCES

[1]Gepts, W. : Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes *14*:619-33, 1965.

[2]Unger, B., Stocks, A.E., Martin, F.I.R., Whittingham, S., and MacKay, I.R.: Intrinsic-factor antibody, parietal-cell antibody and latent pernicious anaemia in diabetes mellitus. Lancet *2*:415-17, 1968.

[3]Blizzard, R. M.: *In* Textbook of Immunopathology. Miescher,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/3/223/348819/25-3-223.pdf by guest on 14 June 2024

TABLE 2

Mean values (± S.E.M.) of T-cell percentages and T-cell absolute numbers per mm.^{3} in peripheral blood of JOD, MOD, and NS

	No. of patients	T-cells %	T-cells per mm.^{3}
NS	18	56.5 (±1.97)	1,261 (±82.35)
JOD	11	38.1* (±4.90)	833† (±155.08)
MOD	21	57.2 (±2.55)	1,026 (±88.05)

*$p < 0.005$ versus NS and MOD
†$p < 0.05$ versus NS

P. A., and Müller-Eberhard, T. H. J., Eds. New York, 1969, p. 547.

[4]Irvine, W. J., Clarke, B. F., Scarth, L., Cullen, D. R., and Duncan, L. J. P.: Thyroid and gastric autoimmunity in patients with diabetes mellitus. Lancet *2*:163-68, 1970.

[5]Bastenie, P. A.: Immunity, autoimmunity, and diabetes. *In* Diabetes. Malaisse, W. J., and Pirart, J., Eds. Amsterdam, Excerpta Medica, 1969, pp. 15-17

[6]Whittingham, S., Mathews, J. D., MacKay, I. R., Stocks, A. E., Unger, B., and Martin, F. I. R.: Diabetes mellitus, autoimmunity and ageing. Lancet *1*:763-66, 1971.

[7]Nerup, J., Andersen, O. O., Bendixen, G., Egeberg, J., Gunnarsson, R., Kromann, H., and Poulsen, J. E.: Cell-mediated immunity in diabetes mellitus. Proc. R. Soc. Med. 67:506-13, 1974.

[8]Bottazzo, G. F., Florin-Christensen, A., and Doniach, D.: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet *2*:1279-83, 1974.

[9]MacCuish, A. C., Barnes, E. W., Irvine, W. J., and Duncan, L. J. P.: Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet *2*:1529-31, 1974.

[10]MacLaren, N. K., Huang, S. W., and Fogh, J.: Antibody to cultured human insulinoma cells in insulin-dependent diabetes. Lancet *1*:997-99, 1975.

[11]Nerup, J., Platz, P., Andersen, O. O., Christy, M., Lyngsøe, J., Poulsen, J. E., Ryder, L. P., Nielsen, L. S., Thomsen, M., and Svejgaard, A.: HL-A antigens and diabetes mellitus. Lancet *2*:864-66, 1974.

[12]Cudworth, A. G., and Woodrow, J. C.: HL-A antigens and diabetes mellitus. Lancet *2*:1153, 1974.

[13]McDevitt, H. O., and Bodmer, W. F.: HL-A, immune-response genes, and disease. Lancet *1*:1269-75, 1974.

[14]Jondal, M., Holm, G., and Wigzell, H.: Surface markers on human T and B lymphocytes. J. Exp. Med. *136*:207-15, 1972.

[15]Augener, W., Cohnen, G., Reuter, A., and Brittinger, G.: Decrease of T-lymphocytes during ageing. Lancet *1*:1164, 1974.

[16]MacCuish, A. C., Urbaniak, S. J., Campbell, C. J., Duncan, L. J. P., and Irvine, W. J.: Phytohemagglutinin transformation and circulating lymphocyte subpopulation in insulin-dependent diabetic patients. Diabetes *23*:708-12, 1974.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/3/223/348819/25-3-223.pdf by guest on 14 June 2024