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Lymphocyte Subpopulations in Insulin-dependent Diabetics with and without Serum Islet-Cell Autoantibodies

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Summary. The lymphocyte subpopulations of 26 insulin-dependent diabetics were studied. Thirteen of them had persistent pancreatic islet-cell serum antibodies (ICA) (mean (± SD) duration of diabetes 11 ± 8 years). The others were ICA-negative (mean duration of diabetes 10 ± 8 years). The mean fasting blood glucose in the week before the lymphocyte count was 1.37 ± 0.45 g/l (two specimens for every patient). As controls 19 healthy volunteers, sex and age matched, were investigated. The T-lymphocyte count was no different in diabetics compared to controls. B-cells were significantly raised ($p < 0.01$) in the ICA-positive group, when tested with anti-human gammaglobulin sera (IgG + IgA + IgM), anti-human IgG and anti-human IgM, while no difference was observed between ICA-negative patients and normal subjects. IgA-bearing lymphocytes were equally raised in both diabetic groups ($p < 0.05$). These data show an altered immunological balance in type IB (autoimmune) diabetes, characterized by an increased number of B-lymphocytes.

Key words: Insulin-dependent diabetes mellitus, T and B lymphocytes, islet-cell antibodies, autoimmunity.

Islet-cell autoantibodies (ICA) have been found in the majority of insulin-dependent diabetics (IDD) of recent onset [1, 2], with the percentage falling rapidly thereafter [1–4]. In patients with transient-ICA, autoimmune diseases are not reported. ICA have also been found in IDD associated with autoimmune endocrinopathies and/or organ-specific autoantibodies [3–7]. In this group the ICA are persistent for many years, and are associated with an increased incidence of the HLA B-8 haplotype [8–10]. ICA are therefore a common marker for two types of IDD, type IA of non-immunological origin, and type IB of possible autoimmune pathogenesis [11].

Previous studies on circulating lymphocytes in IDD have given various results: this may be due to differences in methods, in the selection of patients or in metabolic control at the time of investigation. MacCuish et al. [12] and other groups [13, 14] did not find significant differences in the number of T and B cells between diabetic patients and normal subjects, independent of the metabolic state. There are reports, however, of a fall in the number of T-lymphocytes, dependent on [15] and independent of [16] metabolic conditions. Recently, Müller et al. [17] showed a reduced percentage of high-affinity E-rosette forming cells in IDD in good clinical state, in comparison to healthy controls, but the total T-lymphocyte number was unchanged.

In an attempt to resolve these discrepancies we have studied the lymphocytic subpopulations in IDD, distinguishing persistent ICA-positive and negative subjects.

Materials and Methods

Patients

The main clinical and laboratory findings of the cases studied are summarized in Table 1. We have investigated 26 IDD patients. Thirteen of them had ICA in at least two separate tests, with one year or more between the first and the last specimen. Six were male and seven female, with a mean age of 44 ± 18 (SD) years (range 14–68), and taking insulin 49 ± 19 U/24 h (range 18–85). At the beginning of our study the mean duration of IDD in the ICA-positive group, was 11 ± 8 years (range 2–25). In only two cases was duration under 6 years, and they remained ICA positive at 3 years. Six patients had other autoantibodies (Table 1), two suffered from Graves' disease and one was taking an antithyroid

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Table 1. Features of patients studied

ICA-negative patients	Sex	Age (years)	Duration of IDD	Fasting blood glucose g/l[a]	Insulin U/d	Other drugs	OSA	Other diseases
1	M	27	2 years	1.33	50	–	–	–
2	M	59	6 years	0.90	30	–	–	–
3	M	64	19 years	1.20	75	–	–	–
4	F	72	10 years	1.24	60	–	–	–
5	F	49	6 years	0.77	50	–	–	–
6	F	74	14 years	1.65	70	–	–	–
7	F	57	22 years	1.39	80	–	–	–
8	M	38	2 years	1.31	20	–	–	–
9	M	33	1 year	1.02	75	–	–	–
10	M	33	4 years	1.06	70	–	–	–
11	M	23	12 years	0.56	75	–	–	–
12	F	36	26 years	1.89	75	–	–	–
13	F	64	8 years	1.78	45	–	–	–
mean ± SD		48±17	10±8 years	1.24±0.39	60±19			
ICA-positive patients								
1	M	25	11 years	1.06	40	–	–	–
2	F	60	2 years	1.34	18	Methimazole	TMA	Graves
3	M	68	18 years	1.01	36	–	–	–
4	F	65	6 years	1.84	55	–	THHA+TMA	–
5	M	27	8 years	1.16	85	–	–	–
6	F	14	2 years	2.19	38	–	TMA	–
7	M	29	20 years	1.48	45	–	–	–
8	F	50	8 years	1.50	48	–	–	–
9	M	45	11 years	1.54	50	–	TMA	Graves
10	F	20	9 years	1.61	63	–	TMA	–
11	M	57	25 years	2.45	50	–	–	–
12	F	51	20 years	0.95	28	–	TMA+PCA	–
13	F	68	7 years	1.58	75	–	–	–
mean ± SD		44±18	11±8 years	1.52±0.46	49±19			

[a] mean value of two recent determinations

OSA = organ-specific autoantibodies
ICA = islet-cell autoantibodies
TMA = thyroid microsomal autoantibodies
THHA = thyroglobulin haemoagglutination autoantibodies
PCA = parietal-cell autoantibodies

drug (methimazole 15 mg/d). The 13 ICA-negative IDD (7 males and 6 females; age 48 ± 17 (range 23–74)) had a mean duration of the illness of 10 ± 8 years (range 1–26), with a mean insulin dose of 60 ± 19 U/d. One subject had ICA until the ninth year of illness, but these antibodies had disappeared at the time of our test. None showed other autoantibodies or autoimmune diseases.

The mean fasting blood glucose in all IDD (based on two different specimens from every patient, both drawn in the week before the test) was 1.37 ± 0.45 g/l (1.52 ± 0.46 in the ICA-positive group, 1.24 ± 0.39 in the ICA-negative group).

As controls 19 healthy volunteers, without serum autoantibodies or autoimmune diseases (11 males and 8 females; mean age 40 ± 17 years (range 12–72)), were also studied.

Autoantibody Detection Tests

ICA were detected with the indirect immunofluorescence technique on 4 μm cryostat unfixed sections of 0 group normal human pancreas, as described elsewhere [3]. To detect other autoantibodies we used, with the same technique, thyrotoxic thyroid, hyperplastic adrenal and stomach tissues, obtained from patients at surgery, and rat kidney and liver, according to Roitt [18]. Thyroglobulin antibodies were titrated by the Thymune-T Haemoagglutination Kit (Wellcome) [18].

T-Lymphocyte Count

T-lymphocytes were measured using the E-rosette test with sheep red blood cells [19], stored at 4 °C (diluted 1:2 v/v in Alsever's solution) for not more than two weeks. Briefly, the lymphocytes obtained according to the Böjum method [20] were then incubated for 30 min at 37 °C with iron-carbonyl (15 mg/ml) in order to eliminate phagocytic cells. They were then resuspended in Hank's balanced salt solution (HBSS) at 2×10^6/ml for 10 min at 37 °C in equal volumes. They were then centrifuged at 200 × g for 5 min. The pellet, after standing for 18 h at 4 °C, was gently resuspended and read, taking E-rosette forming cells as those with three or more erythrocytes adhering to the surface.

B-Lymphocyte Count

For B-lymphocyte identification, the cells, suspended at 10×10^6/ml, were incubated in an ice-bath for 30 min with the same volume of fluorescein-conjugated sera: a) anti-human immunoglobulins (IgG + IgA + IgM); b) anti-human IgG; c) anti-human IgA (all from goat) and d) anti-human IgM (from rabbit) (Behringwerke). They reacted specifically to human IgG, IgA and IgM by immunoelectrophoresis. We used them diluted 1:3.

For total Ig (G + A + M) and IgG, cells incubated for 60 min at 37 °C before washing in HBSS and mixing with the antisera were used. These separate steps were performed in order to leave out the so-called L-cells [21] (lymphocytes carrying surface receptors for the Fc fragment of isolated or better aggregated circulating immunoglobulins of the IgG class). With an incubation of 60 min at 37 °C the membrane-adhering Ig were eluted [22–24]. All the suspensions were then washed ×3 in Hank's solution and the final pellets were observed with a Leitz Orthoplan microscope equipped with HBO 100 W lamp and K 455/490 filters.

Statistical Method

All the data obtained from each group were then statistically compared using the Student "t" test.

Results

The total lymphocyte number per microlitre and E-rosette forming cell number, in both absolute and percent terms, are shown in Table 2. There was no difference between the three groups of subjects. The percentage of B-cells is shown in Table 3. The differences between IDD groups were very significant ($p < 0.01$), with a constant increase of B-lymphocytes in ICA-positive patients, with respect to both ICA-negative patients and to controls. Only IgA-cells were equally raised ($p < 0.05$) in both diabetic groups. No other change was observed between ICA-negative patients and normal controls.

Table 2. Lymphocyte count and sheep red blood cell (SRBC) rosettes in diabetic patients (mean ± SD)

	No. of subjects	Lymphocytes/mm³	Total SRBC rosettes %	Total SRBC rosettes/mm³
Insulin-dependent diabetics ICA+ve	13	2231±196[a]	59.7±3.7[a]	1296±167[a]
Insulin-dependent diabetics ICA-ve	13	1977±218[a]	61.6±2.9[a]	1317±155[a]
Normal subjects	19	1974±213	65.9±2.0	1308±134

[a] $p > 0.05$

Table 3. Surface immunofluorescence results using specific Ig class antisera

	No. of patients	Ig (G-A-M)	IgG	IgA	IgM
Insulin-dependent diabetics ICA+ve	13	12.7±1.1[b]	5.2±1.0[b]	0.8±0.2[a]	7.3±0.9[b]
Insulin-dependent diabetics ICA-ve	13	6.9±1.6	3.5±0.6	0.8±0.3[a]	4.0±0.7
Normal subjects	19	5.3±0.5	2.1±0.2	0.3±0.1	4.1±0.5

Results are given as membrane-Ig bearing cells as percent of total lymphocyte number (± SEM)
[a] = $p < 0.05$ and [b] = $p < 0.01$, when compared to normal subjects

Discussion

Reported results on T-lymphocyte numbers in IDD disagree [12–17]. The metabolic compensation [15], the duration of the illness [25] and the patients' age, which is inversely correlated to the T-lymphocyte absolute number [26], are perhaps relevant. Furthermore, the IDD cases previously studied were not homogeneous from a pathogenetic point of view, immunological status differs in the various types of IDD.

Our results indicate a normal number of E-rosette forming cells in type IA and IB of IDD patients. In contrast, we found a raised number of membrane-Ig bearing cells in ICA-positive patients, in comparison with ICA-negative IDD and with normal controls. The presence of ICA among type I diabetic patients for more than three years [3, 27, 28] after the beginning of the disease allows us to classify these patients as belonging to type IB according to Bottazzo and Doniach [11]; this type may be of autoimmune pathogenesis.

It seems relevant that only this group showed an increase both of total B-lymphocyte number and of each B-cell subpopulation. This may represent a confirmation of the altered immunological balance, causing the autoantibody production. We can suppose that the T-cell subpopulations might be altered without a total T-cell number change.

The different results obtained by us by comparison with those previously published in regard to the B-lymphocyte number, might depend on the cases studied. Until now no one has examined, as a selected group, patients with persistent ICA.

As we used antisera consisting of the whole immunoglobulin molecule, we cannot exclude that

the B-cell increase reported above may be due (totally or partly) to a rise of K-cells. These bind the Fc fragment of immunoglobulins. This agrees with the hypothesis that K-cells are increased in ICA-positive patients, supporting recent data [25].

In conclusion, it is possible that IDD of the type IB has another immunological marker besides persistent ICA and other organ-specific autoantibodies with/without autoimmunopathies [11], namely the increased number of circulating B-lymphocytes.

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