Anti-islet Cellular and Humoral Immunity, T-Cell Subsets, and Thymic Function in Type I Diabetes

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SUMMARY

Peripheral lymphocyte subsets were enumerated, using OKT monoclonal sera, in 56 diabetic (43 adults and 13 children) and 20 control subjects. Concomitantly, anti-islet humoral and cellular immunity was tested in vitro and serum thymulin level was measured.

In the newly diagnosed patients (<30 days; 18 cases), the percent of OKT4$^+$ and OKT8$^+$ cells was reduced, the OKT8$^+$ depletion being particularly pronounced in children. Tests for cellular immunity were positive in 83% of the newly diagnosed diabetic subjects and anti-islet cytotoxic antibodies were detected in 50%. The serum thymulin level was decreased in 2 children. Later on in the course of the disease, a marked reduction in OKT3$^+$, OKT4$^+$, and OKT8$^+$ cell percentage was observed, the mean OKT4/OKT8 ratio being normal or lower than normal. The percent of antibody-positive sera rose to 64%, while anti-islet cellular immunity was detectable in 54%. When extrapancreatic manifestations of probable autoimmune nature were present, anti-islet cellular immunity was detected in 100% of cases, accompanied by cytotoxic antibodies in 54%.

Conclusions: (1) the magnitude of T-cell depletion and/or imbalance in diabetic subjects depended mainly on the duration of the disease, (2) anti-islet cellular immunity was the anomaly most frequently detectable, and (3) a decrease in serum thymulin level was infrequently detected. DIABETES 1985; 34:373–79.

Several lines of evidence suggest that autoimmune phenomena directed against islets of Langerhans are present in type I (insulin-dependent) diabetic subjects (reviewed in ref. 1). Autoimmunity is frequently associated with an imbalance of circulating T-lymphocyte subsets,[2-8] which is the most pronounced at the onset of the disease.[2] Similar abnormalities of the thymus-dependent immune system, such as lymphocytopenia and/or T-cell depletion, are noted in diabetic BB rats,[9] *db/db* mice,[10] and some insulinopenic diabetic dogs.[11] The involvement of T-cells in the disease pathogenesis is further suggested by the beneficial effects of antilymphocyte serum,[12] x-radiation,[12] and cyclosporin treatment.[13,14] Lastly, a decrease in the production of the serum thymic factor (FTS or thymulin) was demonstrated in the *db/db* mice concomitantly with T-lymphocytopenia, T-cell imbalance,[15] and anti-islet cellular and humoral immunity. It is the aim of this study to correlate, in 56 patients with type I diabetes, anti-islet autoimmunity, lymphocyte subsets, and serum thymulin level.

SUBJECTS AND METHODS

SUBJECTS

Clinical parameters of the 56 patients under study are shown in Table 1. Patients included 13 children (mean age 9 ± 1 yr, range 1.5–14 yr) and 43 adults (mean age 35 ± 3 yr, range 15–82 yr). Eighteen patients had recently diagnosed disease (<30 days, range 15–30 days) and 38 had longer-lasting disease (1 mo to 40 yr). The recent-onset patients comprised 10 males and 8 females aged 23 ± 4 yr (mean ± SEM, range 1.5–55). The non-recently diagnosed patients included 20 males and 18 females aged 32 ± 3 yr (range 9–82 yr). Eleven diabetic subjects presented with associated extrapancreatic manifestations evocative of autoimmunity: seven had thyroid disease, six had vitiligo (three of them also with thyroid disease), one had rheumatoid arthritis, and one had circulating anti-DNA antibodies. Ten healthy, adult blood donors and 10 healthy children served as controls. It was verified that control subjects were free of personal and familial history of diabetes mellitus and autoimmune diseases. Factors known to interfere with the blood level of lymphocyte subsets were carefully investi-

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Received for publication 25 January 1984 and in revised form 8 October 1984.

TABLE 1
Characterization of patients

Case no.	Sex	Age (yr)	Duration of diabetes (mo)	HbA_1 (%)	Associated extrapancreatic autoimmune phenomena
1	M	1.5	1	14.3	–
2	M	2.5	1	—	–
3	M	6	1	7.1	–
4	F	7	1	13.2	–
5	F	9	1	12.4	–
6	M	10	1	14	–
7	M	12	1	—	–
8	M	21	1	14	–
9	M	24	1	11	–
10	F	25	1	11.8	–
11	F	28	1	15	–
12	F	29	1	8.9	–
13	F	30	1	15	–
14	F	31	1	15	–
15	M	36	1	15	–
16	M	38	1	13	–
17	M	47	1	17	–
18	F	55	1	15	–
19	M	9	2	13.2	–
20	M	9	2	—	–
21	F	10.5	12	15	–
22	M	11	24	7.8	–
23	F	13	60	—	–
24	M	13	24	14.1	–
25	F	15	5	17	–
26	M	16	60	11.9	–
27	M	16	120	11.3	–
28	F	16	12	13	–
29	M	16	9	7.3	–
30	F	16	1.5	19	–
31	M	18	5	7	–
32	F	20	24	11	–
33	F	26	3	15	–
34	M	28	60	14	–
35	M	28	6	15	–
36	F	30	204	14	–
37	M	31	3	14	–
38	M	32	36	18	–
39	M	35	36	13	–
40	M	35	5	15	–
41	F	35	6	—	–
42	F	38	4	13	–
43	M	62	2	19	–
44	M	73	456	11	–
45	F	15	24	—	Anti-DNA antibodies
46	F	18	180	10.6	Hashimito thyroiditis
47	M	34	60	16	Vitiligo
48	F	34	48	10	Graves disease
49	F	35	60	7.2	Vitiligo
50	M	42	96	11	Graves disease
51	M	45	300	19	Vitiligo
52	M	60	120	11	Rheumatoid arthritis
53	F	60	480	12	Vitiligo + myxedema
54	F	71	240	12.5	Vitiligo + myxedema
55	F	71	240	12	Vitiligo + myxedema
56	F	82	180	—	Graves disease

In the upper part of the table (cases 1–18) appear the recent-onset (<1 mo) diabetic subjects. In the middle part (cases 19–44) are the patients with diabetes lasting for more than 1 mo and free of associated autoimmune phenomena. In the lower part of the table (cases 45–56) appear the patients with associated extrapancreatic phenomena. In each of these three parts, patients are classified according to age.

gated in all patients. One diabetic subject had had chicken pox a few weeks before lymphocyte typing. Another one (with rheumatoid arthritis) was treated with low doses of corticoids and aspirin at the time of the study. The metabolic control of diabetes was assessed by glycosylated hemoglobin measurement. White cells and the percent of lymphocytes in peripheral blood were counted in most patients at the time of the study.

METHODS

Lymphocyte subsets. The monoclonal antibodies OKT3, OKT4, and OKT8 were obtained from Ortho Pharmaceutical Corporation (Raritan, New Jersey). These antibodies are directed, respectively, toward the mature peripheral T-cells (OKT3), the helper-inducer T-cells (OKT4), and the suppressor-cytotoxic T-cells (OKT8).[16] Indirect fluorescence assay was performed as previously described.[17] In brief, 10 ml of venous heparinized blood was diluted and layered over a Ficoll-Telebrix density gradient. After centrifugation, the lymphocyte interface layer was washed twice in Hanks' balanced salt solution (HBSS) and the cell count was adjusted to 10–20 × 10^6 cells/ml. Fifty microliters of the lymphocyte suspension was placed in each of three tubes. The tubes were then incubated for 30 min at 4°C, according to the manufacturer's recommendations, with 5 μl of appropriate dilutions of OKT3, OKT4, and OKT8 monoclonal sera. The cells were washed in cold HBSS containing 5% fetal calf serum and 0.2% sodium azide. They were labeled with 5 μl of fluoresceinated goat anti-mouse IgG for 30 min at 4°C, washed twice in cold medium, resuspended gently with a Pasteur pipette, and one drop was examined with a Leitz Orthoplan microscope. Two hundred cells were counted per slide. The results were expressed as the percentage of each T-cell subset with respect to the total number of mononuclear cells present in each field.

Anti-islet immunity. Complement-dependent anti-islet antibodies were detected by a method based on ^{51}Cr release from ^{51}Cr-labeled islet cells.[18] Murine islet cell suspensions prepared in minimum essential medium (MEM Eagle, Flow Laboratories, Rockville, Maryland), modified as previously published,[19] were incubated for 1 h at 37°C with ^{51}Cr sodium chromate (10^6 cells plus 0.1 mCi in 500 μl medium). After washing, cells were resuspended onto microtest plates (5 × 10^3 cells in 100 μl medium) and incubated for 30 min at 37°C with 15 μl of control or test sera, under an air + CO_2 atmosphere. Then, 85 μl of a 1/20 dilution of rabbit complement (Cederlane, Hornby, Ontario) was added and the incubation was continued for a further 30 min. Supernatant aliquots were harvested and counted in a Beckman MK 9000 gamma counter. The percent of ^{51}Cr release was compared with that of distilled water-lysed islet cells.[20] All sera were tested in triplicate. The intraassay variation coefficient was 7% and the interassay variation coefficient was 20%.

Cellular anti-islet immunity was detected by an in vitro insulin suppression test.[19] In brief, murine islet cells prepared in MEM modified as above (5 × 10^3 in 0.1 ml) were incubated in the presence of control or patient lymphocytes (4 × 10^5 cells in 0.1 ml) for 18 h at 37°C in 5% CO_2 humidified air. The wells were washed and supernatants replaced by basal or stimulatory medium containing 16.5 mmol/L glu-

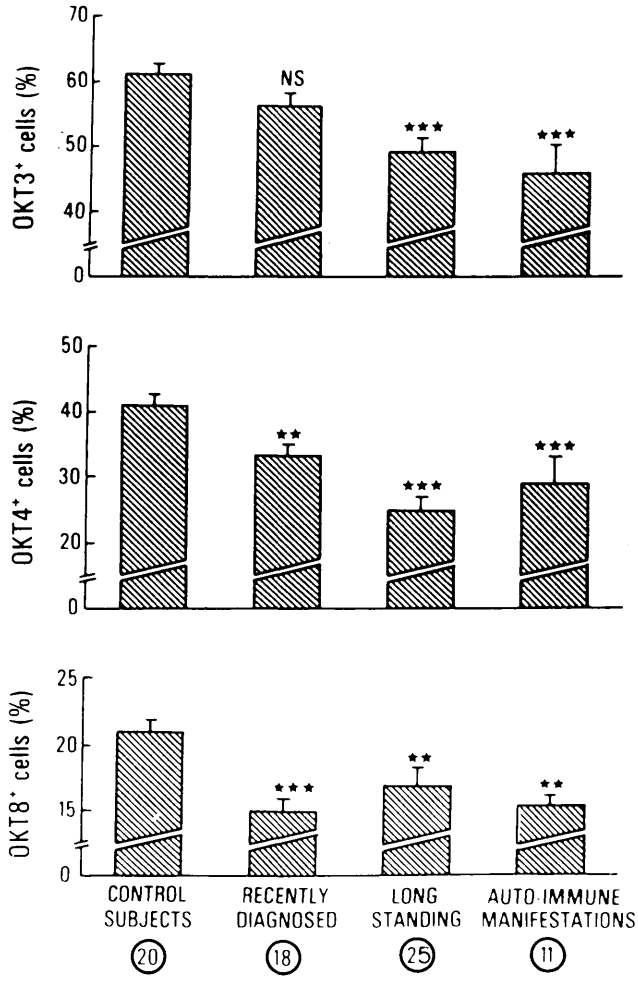

FIGURE 1. Lymphocyte subsets in control subjects and diabetic patients. Results are presented as mean values ± SEM. Numbers of subjects appear at bottom of columns. Statistical significance of differences from control values is denoted by asterisks: **P < 0.01, ***P < 0.001.

cose and 5 mmol/L theophylline (Sigma, St. Louis, Missouri). Plates were kept at 37°C for 5 min and supernatants were collected and rapidly frozen for insulin determination. All lymphocytes were tested in quadruplicate.

Thymulin bioassay. This assay was based on a previously described rosette assay.[21] Briefly, sera were filtered through CF50 Amicon membranes and incubated for 90 min at 37°C with spleen cells from C56BL/6 mice thymectomized (Tx) 10–15 days before the test, and with 10 μg/ml azathioprine (Az), a concentration inhibiting rosette formation in normal mice but not in Tx mice. Rosettes were then formed by centrifugation with sheep red blood cells (Institut Pasteur, Paris) and enumerated in a hematocytometer after gentle resuspension. In the presence of FTS-containing sera, rosette formation was inhibited by Az. The FTS serum level was defined as the highest serum dilution inducing sensitivity to Az inhibition in spleen cells from Tx mice. The normal range is 1/8 to 1/32.

Expression of results and statistical studies. Data are presented as mean values ± SEM. The statistical significances of differences between parametric values were calculated by Student's *t*-test for unpaired values.

The insulin (IRI) secretion from isolated islet cells varied slightly from one experiment to another. For this reason, and to render results comparable, an IRI secretory index was calculated:

$$\frac{(\text{stimulated IRI release}) - (\text{basal IRI release})}{(\text{basal IRI release})} \times 100$$

A normal range was defined as the mean value ± 2SD for the IRI secretory index in the presence of control lymphocytes and, for the ^{51}Cr release, in the presence of control sera plus complement. Patients' lymphocytes and sera were considered abnormal when below (for lymphocytes) or above (for sera) the corresponding cutoffs.

RESULTS

CONTROL SUBJECTS

As shown in Figure 1, in healthy controls, the percent of OKT3⁺ cells (T-lymphocytes) was 61 ± 2% (mean ± SEM, range 48–75), that of OKT4⁺ cells (helper/inducer) was 41 ± 2% (range 30–50), and that of OKT8⁺ (suppressor/cytotoxic) 21 ± 1% (range 11–31). The mean OKT4/OKT8 ratio was 2.10 ± 0.20 (range 1.10–2.30). No difference was noted between adult and child controls regarding the percent of subset lymphocytes.

The ^{51}Cr release from labeled islet cells in the presence of control sera plus complement was 26 ± 1%, versus 25 ± 2% for cells plus complement, in the absence of serum (Figure 2). The upper limit of normal range (mean + 2SD) was 41%. This relatively high value may be partly due to the nonspecific cytotoxicity of some control sera and some complement batches.

The basal and stimulated IRI secretions from dispersed islet cells were, in the presence of control lymphocytes, not different from those measured in the absence of lymphocytes: 2.80 ± 0.40 μU/5000 cells/5 min (versus 2.60 ± 0.47) and 6.90 ± 1.30 μU/5000 cells/5 min (versus 5.60 ± 0.87), respectively, the IRI secretion index being 132 ± 11% versus 124 ± 14 in the absence of lymphocytes. The lower limit of normal range (mean values − 2SD) was 38% (Figure 3).

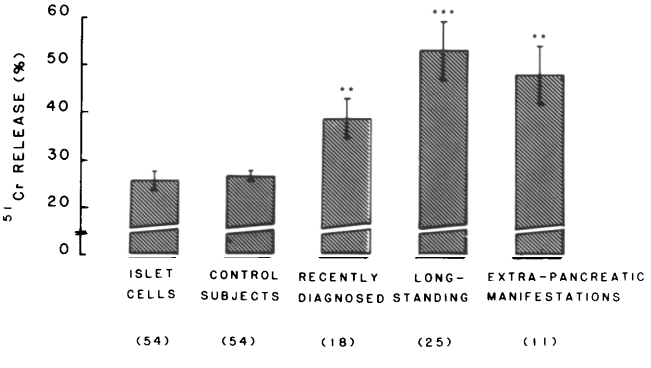

FIGURE 2. ^{51}Cr release from labeled islet cells incubated in the presence of complement (C) alone, and in the presence of C plus control or diabetic sera. Same presentation as in Figure 1.

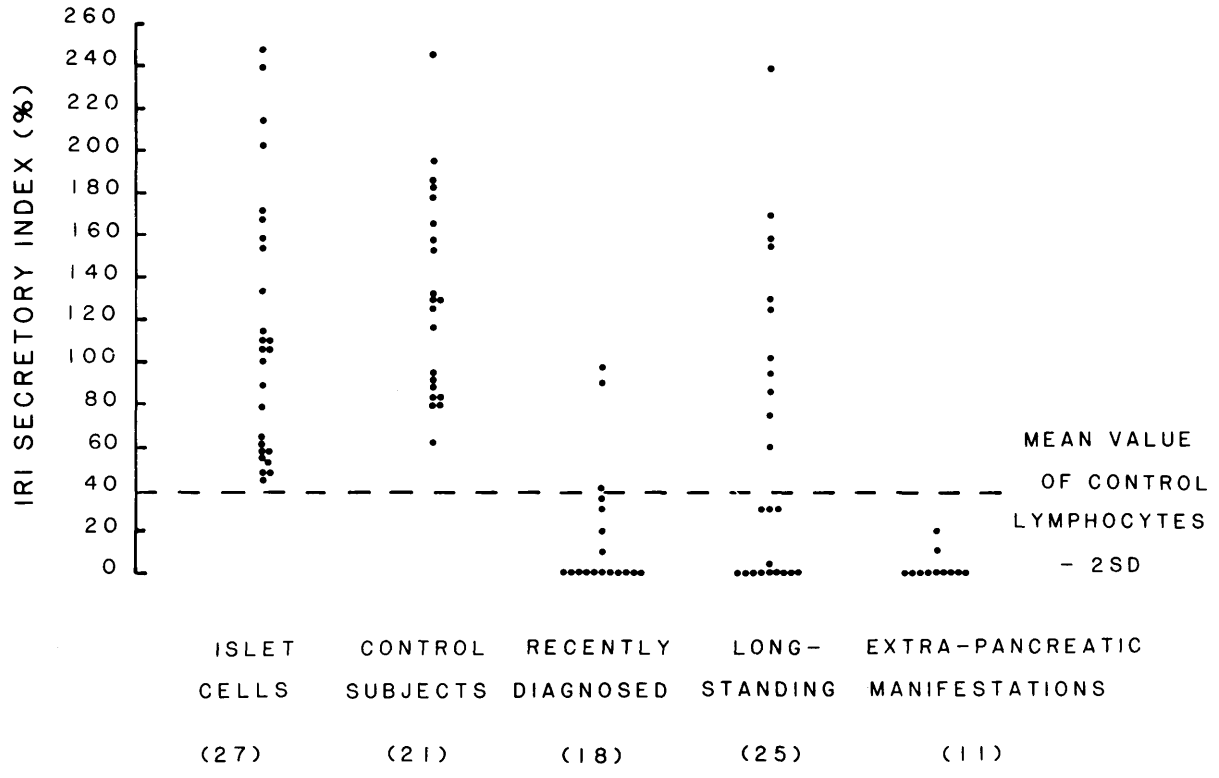

FIGURE 3. Insulin responses to glucose 16.5 mmol/L plus theophylline 5 mmol/L from islet cells incubated alone and in the presence of control and diabetic lymphocytes. Results are expressed as insulin secretory index (as defined in text). Each point corresponds to the mean of four determinations. Horizontal dashed line corresponds to the lower limit of normal range (mean value − 2 SD of control sera).

Thymulin, assayed in child sera, remained in the normal range with the lowest rosette-inhibiting dilution of sera being 1/32 to 1/32.

RECENTLY DIAGNOSED DIABETIC SUBJECTS

When the group was considered as a whole, the mean OKT3 percentage (58 ± 2%) was not different from the control value. However, the percentages of $OKT4^+$ and $OKT8^+$ cells were significantly reduced: 35 ± 2% (versus 41 ± 2%, $P < 0.01$) and 15 ± 1% (versus 21 ± 1%, $P < 0.001$), respectively (Figure 1). The mean OKT4/OKT8 ratio was not significantly modified: 2.55 ± 0.30 (versus 2.10 ± 0.10). The mean ^{51}Cr release from labeled islet cells in the presence of sera plus complement was 38 ± 4% ($P < 0.01$ from controls) as shown in Figure 2. Nine sera, out of eighteen, gave a ^{51}Cr release above the cutoff value of 41%, ranging from 43% to 68%. Incubation of patient lymphocytes with murine islet cells greatly modified the IRI release. The mean basal release was higher than in the presence of control lymphocytes: 5.28 ± 0.63 μU/5000 cells/5 min ($P < 0.001$). The stimulated release was no longer different from basal values, 4.99 ± 1.16 μU/5000 cells/5 min, suggesting that the beta cell response to glucose plus theophylline was suppressed. The mean IRI secretory index was 19 ± 7% versus 132 ± 11% in controls. It was below 2 SD of control value in 15 patients of 18 (being zero in 11) and in lower normal range in the 3 others (40%, 90%, and 98%, respectively) (Figure 3). This scattering of beta cell responses in the presence of patients' lymphocytes accounts for the fact that the mean stimulated IRI release was not significantly different from the corresponding control value.

LONG-STANDING DIABETIC SUBJECTS

Patients free of associated extrapancreatic manifestations. The mean level of $OKT3^+$ cells was significantly reduced (50 ± 2% [range 31–68, $P < 0.001$]) as was that of

TABLE 2
White blood cells, total lymphocytes, and lymphocyte subset counts in the adult controls and in the diabetic patients (per mm^3)

	Control adult subjects	Recently diagnosed patients	Long-standing diabetes	Extrapancreatic autoimmune manifestations
Total WBC	6950 ± 370	7500 ± 1300	6600 ± 550	8300 ± 1300
Total lymphocytes	2200 ± 140	2200 ± 340	2200 ± 130	2300 ± 210
OKT3	1340 ± 90	1260 ± 240	920 ± 50	1160 ± 160
OKT4	900 ± 60	630 ± 145	440 ± 50	800 ± 150
OKT8	460 ± 30	420 ± 120	370 ± 30	380 ± 40

Results are presented as mean values ± SEM. Number of subjects studied was 10–20 in each group.

OKT4$^+$ cells (26 ± 2% [range 9–54, $P < 0.001$]) and that of OKT8$^+$ cells (17 ± 1% [range 8–29, $P < 0.01$]) as shown in Figure 1. The OKT4/OKT8 ratio was diminished: 1.63 ± 0.21 ($P < 0.05$). The mean ^{51}Cr release from labeled islet cells was higher than normal: 52 ± 6% ($N = 25$, $P < 0.001$, Figure 2). Sera were positive (above 2 SD of normal) in 16 patients (ranging from 49% to 100% of chromium release), and negative in the 9 others. The lymphocytes deeply modified the IRI release. The basal release was higher than control values: 5.13 ± 0.61 μU/5000 cells/5 min ($P < 0.001$ from controls) and the addition of the stimulatory medium induced no significant beta cell response: 7.47 ± 1.38 μU/5000 cells/5 min (NS from controls). The mean IRI secretory index was 63 ± 14% (versus 132 ± 11% in controls, $P < 0.001$), the individual values being below 2 SD of normal in 13 patients and zero in 9 patients (Figure 3).

Patients with associated extrapancreatic manifestations. The anomalies of OKT-defined cells were very similar to those detected in previous groups with depletion of all T-cell subsets (Figure 1). The percent of OKT3$^+$ cells was 46 ± 4% (range 17–65, $P < 0.001$), that of OKT4$^+$ cells was 29 ± 4% (range 5.5–45, $P < 0.001$), and that of OKT8$^+$ cells was 16 ± 2% (range 9–36, $P < 0.01$). The OKT4/OKT8 ratio was higher than normal in 3 patients.

The mean complement-dependent toxicity of sera to labeled islet cells was increased: 47 ± 6% ($P < 0.01$, Figure 2), 6 of the sera of 11 being cytotoxic. The lymphocyte-induced suppression of beta cell response to stimuli in vitro was strongly positive in all patients. Again, the basal insulin release was higher than normal: 4.70 ± 0.90 μU/5000 cells/5 min ($P < 0.001$ from controls). No beta cell response to glucose plus theophylline was detectable: 4.00 ± 0.79 μU/5000 cells/5 min (NS from corresponding value in controls). The mean secretory index was 3 ± 2% (versus 132 ± 11% in controls); it was zero in 9 patients and below 2 SD of normal value in all patients (Figure 3).

TABLE 3
Anti-islet humoral immunity (^{51}Cr release test), IRI secretory index, and OKT4/OKT8 ratio in diabetic patients

Case no.	OKT4/OKT8 ratio	^{51}Cr release test (%)	IRI secretory index (%)
1	4.80*	63*	0*
2	3.10*	68*	0*
3	1.60	31	10*
4	3.70*	39	0*
5	1.80	43*	0*
6	1.27	20	20*
7	2.90*	55*	0*
8	1.33	46*	98
9	1.40	50*	0*
10	1.78	48*	90
11	2.92*	13	35*
12	5.50*	10	0*
13	2.26	23	0*
14	1.32	19	0*
15	2.26	58*	30*
16	3.92*	25	0*
17	0.94	61*	40
18	3.19*	15	0*
19	0.70	54*	0*
21	1.00	59*	30*
22	1.81	21	0*
23	2.60*	58*	125
24	1.50	38	0*
25	3.25*	19	0*
26	2.20	51*	59
27	1.20	69*	0*
28	0.60	87*	30*
29	0.90	100*	0*
30	1.53	11	159
31	2.30	14	102
32	1.30	60*	0*
33	2.02	13	
34	1.70	93*	130
35	0.75	88*	30*
36	1.50	49*	170
37	1.00	57*	157
38	0.50	88*	95
39	1.10	64*	240
40	2.00	12	0*
41	2.40	17	75
42	5.40*	30	0*
43	0.90	88*	7*
44	0.65	57*	86
45	1.10	56*	0*
46	3.30*	36	0*
47	2.50	29	10*
48	2.10	15	0*
49	0.60	81*	20*
50	1.71	55*	0*
51	2.40	32	0*
52	1.90	55*	0*
53	0.40	81*	0*
54	3.40*	27	0*
56	3.75*	48*	0*

*Asterisks denote values out of normal range.

WHITE CELL COUNTS

Peripheral cell counts were measured in controls and in most patients of each diabetic group at time of T-cell typing. Children displayed higher lymphocyte counts than did adults. No major difference was noted between control and diabetic groups regarding white cells and total lymphocyte counts (Table 2). No increase in monocyte counts was detected in patients with a low OKT3 percent.

THYMULIN (FTS) PLASMA LEVEL

Thymulin was evaluated in 11 children and 3 adults. Only two displayed low titers (1/4 in cases 2 and 5). The two corresponding sera were strongly cytolytic for islet cells and the corresponding lymphocytes suppressed insulin release. A higher-than-normal OKT4/OKT8 ratio was found in one patient (case no. 2). The thymulin level was normal in all other young diabetic subjects.

DISCUSSION

The recently diagnosed diabetic subjects were characterized by OKT4$^+$ and OKT8$^+$ cell depletions, the OKT8$^+$ depletion being particularly pronounced in children. The mean OKT4/OKT8 ratio was not significantly higher than normal, but abnormally high individual values were detected mostly in this group. Islet reactive lymphocytes were detected in all recent-onset children and in 8 of 11 adults, while sera were cytotoxic to islets in only 4 children and 5 adults. A decreased thymulin concentration was found in 2 children. When the clinical disease had lasted for more than 1 mo, a concomitant marked depletion in OKT3, OKT4, and OKT8

cells was observed with normal or decreased OKT4/OKT8 ratios. A clear-cut difference appeared according to the presence or not of associated extrapancreatic autoimmune manifestations: lymphocytes from all patients with autoimmune phenomena suppressed insulin release in vitro, whereas the sera were cytotoxic in 6 patients. A higher-than-normal OKT4/OKT8 ratio was detected in 3 cases. In the absence of associated extrapancreatic autoimmune manifestations, a high OKT4/OKT8 ratio was detected in only 3 of 25 patients; lymphocytes suppressed insulin release in 13 cases and sera were cytotoxic in 16 patients.

Lymphocyte subsets have already been studied in diabetic subjects either by use of monoclonal antibodies directed toward T-cell markers,[2-6] or by using a functional test for con-A-activated suppressor lymphocytes.[7,8] $OKT3^+$ and $OKT4^+$ subsets were decreased in type I diabetic subjects, with disease lasting for more than a few weeks, in some[2] but not in all studies.[4] An increase in the cytotoxic subset (as detected by the monoclonal UCTH4) was detected only in very recently diagnosed diabetic subjects (<3 days).[2] A decrease in suppressor lymphocytes was found in such patients using either OKT monoclonals[2-4] or functional tests.[7,8]

Finally, there is an irregular increase in helper cells and a decrease in suppressor lymphocytes in type I diabetes. A high OKT4/OKT8 ratio was detected in 14 patients only, including 8 recent-onset and 3 clinically autoimmune patients; all were positive for cellular and/or humoral anti-islet immunity (Table 3). The present cross-sectional study may have blunted some time-related variations in T-cell imbalance. This imbalance may be transient and disappear after the acute phase of insulitis,[2] which may well be missed.[22] Additionally, surface marker differences may not always correspond to differences in lymphocyte function. We have found, as have others,[2,3] a T-cell ($OKT3^+$) lymphopenia, affecting also the $OKT4^+$ subset in long-standing, type I diabetes. This finding is reminiscent of the T-cell lymphopenia oberved in diabetic BB rats,[9] some insulin-dependent dogs,[11] and *db/db* mice,[15] albeit less clear-cut than in each of these animal models. In the diabetic *db/db* mice, the T-lymphopenia affects the suppressor-cytotoxic subset, with an increased Ly^1/Ly^2 ratio. The reason for the T-cell imbalance (when present) and for the OKT3 depletion in long-standing diabetes remains unclear. The hypothesis that a thymic deficiency causes the T-cell anomalies has been suggested by studies performed in the *db/db* mouse.[10] In the present study, however, only two children displayed a decreased thymulin level (associated in one case with an abnormally high OKT4/OKT8 ratio). One may speculate about the contribution of this T-cell imbalance to triggering anti-islet immunity. The recent observation of higher-than-normal OKT4/OKT8 ratios returning to normal range in cyclosporin-treated patients, concomitantly with the occurrence of remission, is consistent with this hypothesis.[23]

The large proportion of patients with islet cell reactive lymphocytes in present study must be emphasized. Patient lymphocytes modified the IRI secretion in 70% of the whole group, with a higher incidence in recent-onset diabetic patients (83%) and in patients with extrapancreatic phenomena (100%), while control lymphocytes did not. The beta cells no longer responded to stimuli, while the basal IRI release was paradoxically enhanced. This latter phenomenon may be due either to a cytolytic passive hormone leakage, or to a yet-unexplained stimulation. The large proportion of islet cell reactive lymphocytes in type I diabetic subjects is in accordance with several lines of evidence that suggest a predominant role for lymphocytes in the pathophysiology of insulitis.[22] The xenogenic origin of the target cells renders the mechanistic interpretation of the test less clear than desirable.[24] However, the results were remarkably consistent with previous studies in human patients,[19] and in diabetic dogs[11] and mice.[10] The cells responsible for the suppression of IRI release in vitro have been shown to be T-cells in diabetic patients[25] and in *db/db* mice.[10]

Consistently, in recent-onset diabetic subjects treated with cyclosporin, this test became progressively negative and was concomitant with the occurrence of remission.[23]

By contrast, sera were toxic to islets only in 50–64% of patients according to the groups, a percentage very similar to that previously obtained by another technique.[26] Peak concentrations of circulating antibodies toxic to islet cells have been detected, in the diabetic BB rat, before or shortly after the occurrence of diabetes.[27] Wide fluctuations in circulating ICA concentrations can occur in humans.[28] Thus, while elevated OKT4/OKT8 ratios were somewhat associated with the recent onset of the disease, the test for anti-islet cellular immunity may be the most frequently positive, particularly in recent-onset and in clinically autoimmune patients.

ACKNOWLEDGMENTS

We express our gratitude to Hélène Cohen for expert technical assistance.

This work was supported by grants from UER Xavier Bichat and the Ministère pour l'Industrie et la Recherche.

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