# Complement-fixing Islet Cell Antibodies from Some Diabetic Patients Alter Insulin Release In Vitro

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**SUMMARY**

**To explore humoral immunity in insulin-dependent diabetic (IDDM) patients, we studied insulin release from isolated mouse islets stimulated by glucose + theophylline after incubation with the sera of these patients and complement. Eleven of 21 IDDM sera suppressed the stimulated insulin release while the arginine-stimulated glucagon release remained unchanged. Morphologic evidence and the trypan-blue exclusion test suggested that the suppression of insulin release was due to a cytotoxic effect of the sera.**

**No beta-cell inhibition or morphologic damage was detectable in the presence of sera from 30 healthy subjects, 8 non-insulin-dependent diabetic patients, and 5 nondiabetic patients with autoimmune diseases. Beta-cell inhibition by IDDM sera was not observed when complement was omitted. After serum fractionation, the cytotoxic potency of IDDM sera was located in the immunoglobulin G fraction. Using human islets, insulin release was suppressed by 3 of 6 IDDM sera.**

**Complement-dependent cytotoxicity was found in 1 of 5 recent-onset IDDM patients and 11 of 16 IDDM patients with autoimmune phenomena. It was associated in all cases with the presence of islet cell antibodies as detected by immunofluorescence, and with the presence of circulating lymphocytes which suppressed insulin release in vitro. Complement-fixing antibodies may contribute to the selective beta-cell damage in IDDM. DIABETES *30*:1051–1057, December 1981.**

Sera from insulin-dependent diabetic patients (IDDM) contain islet cell antibodies (ICA) detectable by indirect immunofluorescence techniques.[1–4] Since some of these ICA are not strictly specific for one cell type in the islets,[2] their role in the specific destruction of beta-cells remains dubious. Other ICA, on the contrary, react specifically with membrane antigens of normal[5] or insulinoma beta-cells.[6] It has been shown that complement-fixing ICA (CF-ICA) may be more closely related to the clinical onset of IDD than are conventional ICA.[7] CF-ICA involvement in islet cell injury was suggested by morphologic criteria[8] and by investigations using the $^{51}Cr$ release test where beta-cells appeared selectively altered.[9] However, there is still no evidence for a specific suppression of insulin (IRI) release from islets exposed to complement-dependent ICA. We have previously suggested that circulating lymphocytes from IDDM patients specifically suppress IRI release from islet cells in vitro.[10]

The aim of the present work is to assess the ability of IDDM sera to suppress IRI release in vitro, in the presence of complement.

## MATERIALS AND METHODS

**Islets.** Pancreatic islets were prepared from DBA/2 adult female mice (CNRS, Orléans, France), as described by Lacy and Kostianovsky.[11] Five pancreases were minced and treated with 15 mg collagenase (150 mU/mg) (Boehringer Mannheim, West Germany) in 3 ml of calcium-free magnesium-free balanced salt solution (CMF) containing 15% fetal calf serum (FCS). After manual shaking at 37°C for 4–5 min, the islets were picked up manually under a dissecting microscope and transferred to Petri dishes set on ice. Ten islets of uniform size and shape were then suspended into each well of Falcon microtest plates (Becton-Dickinson, Oxnard, California) in MEM Eagle Medium (Flow Laboratories, Rockville, Maryland) supplemented with 10% FCS, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 0.814 mg/L nonessential aminoacids, 100 $\mu$g/ml streptomycin, and 100 $\mu$U/ml penicillin. This "basal" medium, containing 5.5 mmol/L glucose, was equilibrated with air + $CO_2$ (95%–5%). Final pH was 7.4.

In some instances, human islets were prepared similarly; the pancreatic tissue was obtained from a transplant kidney donor (HLA type A1, A3, B17, BW35, Dr6-7). Pancreatic fragments (approx. 6 g each) were injected with Hanks'

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buffer, minced, and shaken for 30 min in the presence of 40 mg collagenase. Islets were then collected as described above.

**Assessment of islet viability.** Plates containing islets were kept at 37°C in the basal medium equilibrated with 5% $CO_2$, for 18 h. Islets were then washed and each well received 250 μl of basal medium for 30 min, then "stimulatory" medium for 30 min. A medium consisting of supplemented MEM Eagle containing 16.5 mmol/L glucose + 5 mmol/L theophylline (Sigma, St. Louis, Missouri) was chosen as standard stimulatory medium for insulin release. Media containing 5.5–20.0 mmol/L glucose + 5 mmol/L theophylline were also tested for determination of maximal IRI secretory response.

In some instances, islets were tested for glucagon release. Each well received 250 μl of either basal medium (5.5 mmol/L glucose) or stimulatory medium (5.5 mmol/L glucose + 20 mmol/L arginine). Supernatants were collected at the end of the 30-min test period and immediately frozen for insulin or glucagon determination. All experiments were performed in quadruplicate.

**Sera.** Human sera were collected from 21 IDDM patients (Table 1), 30 healthy subjects, 8 non-insulin-dependent diabetic patients (NIDDM), and 5 nondiabetic patients having manifestations of autoimmune disease. All sera were heat-inactivated for 30 min at 56°C. Some sera were submitted to fractionation by a 40% (w/v) $(NH_4)_2SO_4$ solution in water followed by chromatography on DEAE cellulose $DE_{52}$ (Whatman, England) and globulins were resuspended in a 9‰ saline solution at a concentration of 15 mg/ml.

Conversely, IgG-free sera were prepared by filtration through a column of sepharose conjugated to anti-human IgG (Miles Laboratories, Elkhart, Indiana). The globulin solutions and the IgG-free sera were tested by immunoelectrophoresis using anti-globulin and anti-IgG sera (Miles Laboratories). Serum aliquots were preincubated for 24 h with mouse islets (50 islets/100 μl serum) for adsorption of ICA. Pooled guinea pig serum, stored at −80°C, was used as a source of complement.

**Functional cytotoxic assay.** Freshly isolated islets were incubated for 18 h at 37°C in basal medium, then washed and incubated (10 islets/well) with 50 μl basal medium (FCS-free) + 80 μl test serum or IgG solution, for 2 h. Twenty microliters of complement was added 3 times at 1-h intervals. Eighteen hours later, 140 μl supernatant was removed. Islets were washed and incubated successively with basal and stimulatory media, 30 min for each incubation. In some instances, heat-inactivated guinea pig serum was used in place of complement.

**Determination and expression of results.** Insulin was determined by radioimmunoassay[12] using charcoal for the separation of free and bound hormone, purified rat insulin as a standard, porcine $^{125}I$ insulin as a tracer (CNTS, Paris, France), and a guinea-pig anti-porcine serum (gift of Dr. Kervran, Paris, France). Glucagon was assayed as previously described.[13,14] Islet function was assessed by the net insulin or glucagon release (μl/islet/min and pg/islet/min, respectively) in the presence of basal or stimulatory medium. This was obtained by subtracting the prestimulatory release (i.e., the amount of insulin or glucagon present in

TABLE 1
Clinical characterization of IDDM patients

| Case no. | Sex* age (yr) | Time since diagnosis of IDDM | Duration of insulin therapy | Associated autoimmune phenomena† |
|---|---|---|---|---|
| | | (Days) | (Days) | |
| 1 | M/17 | 10 | 7 | — |
| 2 | M/29 | 8 | 1 | — |
| 3 | F/9 | 7 | 3 | — |
| 4 | F/25 | 15 | 8 | — |
| 5 | F/11 | 15 | 15 | — |
| | | (Yr) | (Yr) | |
| 6 | F/34 | 2 | 2 | Graves' disease |
| 7 | M/37 | 5 | 2 | Graves' disease |
| 8 | F/60 | 20 | 9 | Graves' disease |
| 9 | M/25 | 1.5 | 0.2 | CAH |
| 10 | F/78 | 20 | 18 | Pernicious anemia |
| 11 | F/70 | 20 | 15 | Hashimoto's thyroiditis |
| 12 | F/68 | 40 | 39 | Vitiligo |
| 13 | F/68 | 20 | 2 | Vitiligo |
| 14 | F/16 | 2 | 2 | Hashimoto's thyroiditis |
| 15 | M/40 | 6 | 5 | Graves' disease |
| 16 | F/64 | 16 | 9 | Myxoedema |
| 17 | F/25 | 5 | 4 | Myasthenia |
| 18 | F/88 | 20 | 20 | Myxoedema |
| 19 | F/31 | 5 | 4 | Vitiligo |
| 20 | F/67 | 17 | 10 | Pernicious anemia |
| 21 | F/54 | 1 | 0.7 | Graves' disease |

* M = male; F = female.
† None of the patients received immunosuppressive treatment, except one (case 9) who was receiving corticoids because of a chronic active hepatitis (CAH).

wells before the test period) from the amount present at the end of the test period:

Net basal release = (release in basal medium) − (prestimulatory release).

Net stimulated release = (release in stimulatory medium) − (prestimulatory release).

Results were expressed as mean values ±SEM. The mean of a quadruplicate determination has been entered into the statistical evaluation as one experiment.

**Morphologic study of islets.** After the functional test, morphologic damage of the islets was evaluated. A 0.4% trypan-blue solution was added to the wells, as described by Rittenhouse et al.[8] Five minutes later, cell damage was assessed by phase-contrast microscopy.

**Immunofluorescence (IFL).** Sera were analyzed for ICA using fresh postmortem human pancreas of blood group O kidney transplant donors.[2] Non-species-specific ICA were tested on DBA/2 mouse pancreas. Classic IFL was performed on 5-$\mu$m-thick cryostat sections of unfixed frozen tissue. Screening tests were performed with fluorescent rabbit anti-human globulins (anti-IgG, IgA, IgM) and with fluorescent anti-mouse IgG. Sera showing fluorescence on human pancreas were further tested with specific antisera (anti-IgG, IgA, IgM). Titers were established with serial dilutions using specific anti-IgG. Complement fixation was tested in a three-layer technique using fresh normal human antiserum as a source of complement and anti-$\beta$1C. All immunofluorescent conjugates were obtained from Dakopaths (Copenhagen, Denmark).

Sections of human adrenal gland and rat stomach were used for IFL detection of adrenal and gastric parietal cell antibodies. Thyroglobulin and thyroid microsomal antibodies were assessed with commercial hemagglutination kits (Welcome Reagents, Beckenham, England) and were considered positive at dilutions of 1/10 and 1/100, respectively. All IDDM sera were tested for insulin antibodies at the dilution of 1–10 as described by Yalow and Berson,[15] using $^{131}$I insulin as tracer in the presence or absence of an excess (0.75 $\mu$U/ml) of unlabeled human insulin.

## RESULTS

### INSULIN RELEASE

**Influence of complement and normal human sera.** Basal IRI release in the presence of 5.5 mmol/L glucose was 0.30 ± 0.05 $\mu$U/islet/min. Following the addition of 16.5 mmol/L glucose + 5 mmol/L theophylline, IRI release significantly increased to 1.05 ± 0.15 $\mu$U/islet/min ($P < 0.001$). IRI release also increased significantly after the addition of complement: basal IRI release was 0.32 ± 0.1 $\mu$U/islet/min and stimulated IRI release was 1.09 ± 0.14 $\mu$U/islet/min. After incubation with control human sera, mean basal IRI release was 0.32 ± 0.03 $\mu$U/islet/min and mean stimulated release was 1.00 ± 0.07 $\mu$U/islet/min. These values were not statistically different from those obtained in the absence of human sera. A significant beta-cell response to 16.5 mmol/L glucose + theophylline was observed in the presence of each of the 30 control sera (Figure 1A). This response was not maximal and a higher IRI release was obtained in the presence of 20.0 mmol/L glucose + 5 mmol/L theophylline: 1.25 ± 0.08 $\mu$U/islet/min.

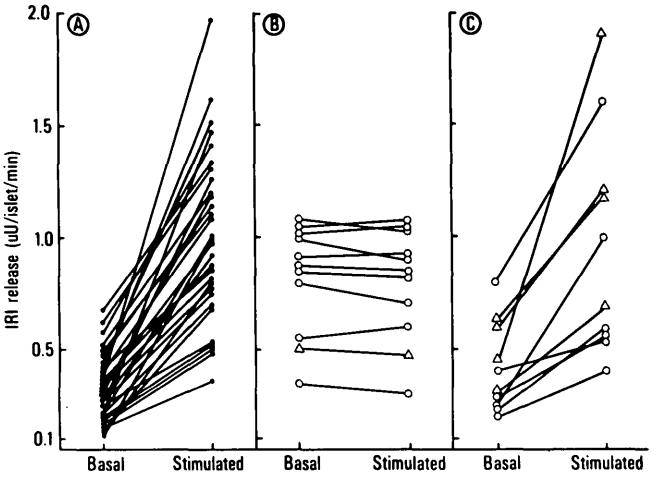



**FIGURE 1. Insulin release from mouse islets, in the presence of control or IDDM sera, and complement. Each point indicates basal (5.5 mmol/L glucose) or stimulated (16.5 mmol/L glucose + 5 mmol/L theophylline) IRI release from islets incubated with control sera (A), suppressive IDDM sera (B), and nonsuppressive IDDM sera (C). Each line joining a basal and a stimulated release corresponds to one individual from a control subject (●), a recent-onset IDDM patient (△), and an IDDM patient with autoimmune disease (○).**

**Influence of IDDM sera: IDDM sera + complement.** In the presence of 11 out of the 21 IDDM sera, stimulated IRI release was not statistically different from basal release (cases 3, 6, 7, 10, 11, 13, 16, 17, 18, 20, 21) (Figure 1B). Mean basal IRI release for this subgroup was higher than in the control group: 0.82 ± 0.09 $\mu$U/islet/min ($P < 0.05$). This was due to a high basal IRI release in the presence of 8/11 IDDM sera. No further increase in IRI release was observed in the presence of each of these 11 IDDM sera when islets were exposed to the standard stimulatory medium (16.5 mmol/L glucose + theophylline): 0.81 ± 0.09 $\mu$U/islet/min. Furthermore, at variance from controls no supplementary IRI release occurred in the presence of 20.0 mmol/L glucose + theophylline: 0.90 ± 0.08 $\mu$U/islet/min.

The 10 other IDDM sera did not suppress the stimulated IRI release, which was statistically higher than basal release ($P < 0.001$) (Figure 1C). In this second IDDM subgroup, mean basal and stimulated values were no different from corresponding values in the control group: 0.41 ± 0.06 $\mu$U/islet/min and 0.97 ± 0.09 $\mu$U/islet/min, respectively. Effects of individual IDDM sera in this subgroup were not distinguishable from effects of individual control sera. In spite of a high basal IRI release with 3/10 sera, a higher IRI release occurred in the presence of stimulatory media.

Based on this heterogeneous response to stimulation, these two subgroups of IDDM sera were termed suppressive and nonsuppressive. Most suppressive sera (10/11) were from IDDM patients having autoimmune phenomena (cases 6, 7, 10, 11, 13, 16, 17, 18, 20, 21). The sera of 1 of 5 recent-onset IDDM patients was suppressive (case 3). Among the 10 nonsuppressive sera, 6 were obtained from IDDM patients with autoimmune diseases, and 4 from patients who had recent-onset IDDM but were free of detectable autoimmune manifestations.

**IDDM sera, in the absence of complement.** When complement was omitted, mean basal IRI release (0.41 ± 0.05 $\mu$U/islet/min) rose, after stimulation to a mean of 0.98 ± 0.9 $\mu$U/islet/min ($P < 0.001$). These values are not different from

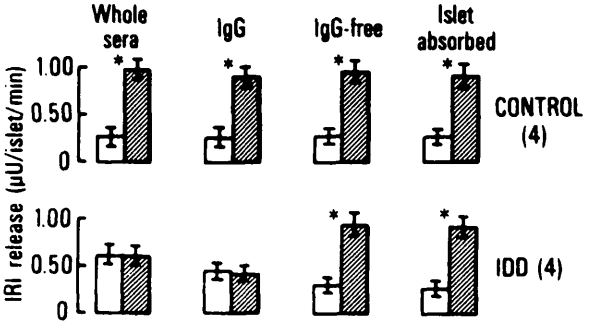



**FIGURE 2. Influence on IRI release of control and IDDM sera, before and after IgG fractionation and islet absorption. Open bars, basal IRI release (5.5 mmol/L glucose). Black bars, stimulated IRI release (16.5 mmol/L glucose + 5 mmol/L theophylline). Number of cases in parentheses. Results are presented as mean values ±SEM. The asterisk denotes a statistically significant difference between basal and stimulated IRI release ($P < 0.001$).**

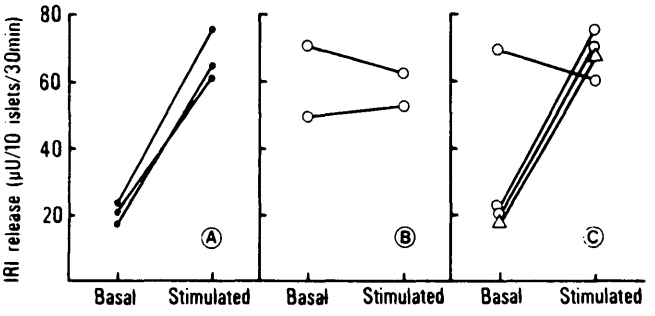



**FIGURE 3. Insulin release from human islets incubated in the presence of complement, with control sera (A), IDDM sera that suppressed mouse IRI release (B) and did not suppress mouse IRI release (C). Sera from recent-onset IDDM patients are shown by triangles; sera from IDDM patients with autoimmune diseases are shown by circles. The presentation is the same as in Figure 1.**

those in the control group. In particular, none of the 11 sera that were suppressive in the presence of complement inhibited IRI release in the absence of complement. Furthermore, in the presence of heat-inactivated guinea-pig serum, no suppression of IRI release was observed with suppressive IDDM sera: mean basal IRI release was 0.44 ± 0.06 μU/islet/min and mean stimulated IRI release was 0.99 ± 0.07 μU/islet/min ($P < 0.001$).

**Fractionated and islet-absorbed sera (Figure 2).** Immunoglobulins IgG prepared from four suppressive IDDM sera (cases 6, 11, 17, 20) inhibited the beta-cell response to glucose + theophylline as well as the corresponding whole sera. IgG from one IDDM serum (case 8) suppressed IRI release at a concentration of 35 mg/ml while whole serum and IgG at a concentration of 15 mg/ml did not. IgG from control sera did not inhibit stimulated IRI release.

IgG-free fractions prepared from suppressive sera (cases 6, 11, 17, 20) lost their suppressive effect. Similarly, after preincubation with islet, the suppressive effect of the sera was lost.

**Influence of NIDDM sera + complement.** In the presence of each of the eight NIDDM sera, a significant beta-cell response to glucose + theophylline was observed: mean basal IRI release was 0.40 ± 0.07 μU/islet/min and rose after stimulation to a mean of 0.99 ± 0.10 μU/islet/min ($P < 0.001$). These values were not different from the corresponding values in the control group.

**Influence of sera from nondiabetic patients with autoimmune diseases.** In the presence of each of the five sera from nondiabetic patients with autoimmune diseases, the beta-cell response to stimulation was not different from that observed in the control group (mean basal IRI release 0.32 ± 0.05 μU/islet/min, mean stimulated release 1.03 ± 0.10 μU/islet/min) ($P < 0.001$).

**IRI release from human islets (Figure 3).** Three control sera were incubated with human islets. In each case, stimulated IRI release was higher than basal release ($P < 0.001$) (Figure 3A). In the presence of two IDDM sera that had been found suppressive for mouse islets, the beta-cell response to glucose + theophylline was inhibited (cases 18, 20) (Figure 3B). Out of 4 IDDM sera which had been found nonsuppressive for mouse islets (cases 1, 9, 14, 19), one suppressed the human beta-cell response to glucose + theophylline (case 19) while the three others did not (Figure 3C). As already noted after mouse islet incubation, the suppressive sera caused an increase of basal IRI release.

GLUCAGON RELEASE (FIGURE 4)

In the presence of control sera, basal glucagon release was 0.73 ± 0.08 pg/islet/min. In the presence of 20 mmol/L arginine, the stimulated glucagon release rose to 1.77 ± 0.09 pg/islet/min ($P < 0.001$). After incubation with 4 IDDM sera that suppressed IRI release (cases 6, 10, 13, 20), both basal and stimulated glucagon releases were not significantly different from controls: 0.75 ± 0.06 pg/islet/min and 1.63 ± 0.06 pg/islet/min, respectively.

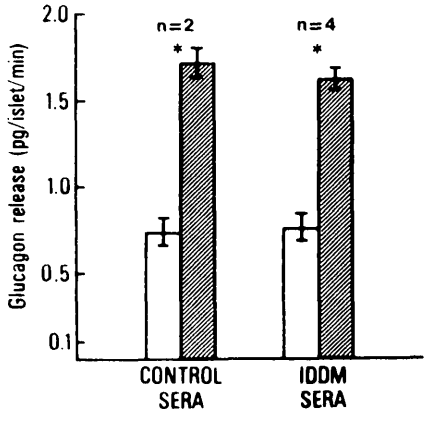
MORPHOLOGIC STUDY OF ISLETS

No detectable morphologic alteration of islets was observed in the presence of complement alone, control sera plus complement, or NIDDM sera plus complement. Islets remained regular in shape and stained cells were infrequent.

By contrast, islets incubated with the 11 suppressive IDDM sera in the presence of complement were grossly modified. Most islets were disrupted and numerous free cells were scattered around them. Islets were deeply stained by trypan blue.

In the absence of complement these 11 IDDM sera did not

**FIGURE 4. Glucagon release from mouse islets, in the presence of control or "insulin-suppressive" IDDM sera, plus complement. Open bars represent basal glucagon release (5.5 mmol/L glucose) and black bars represent stimulated release (5.5 mmol/L glucose + 20 mmol/L arginine). Results are presented as mean values ±SEM. Number of sera appears in parentheses. The asterisk denotes a statistically significant difference between basal and stimulated release ($P < 0.001$).**



display such morphologic clues of cytotoxicity. The nonsuppressive IDDM sera did not cause any detectable morphologic damage to the islets, in the presence or in the absence of complement.

IMMUNOFLUORESCENCE STUDY OF SERA (TABLE 2)

Using human or mouse islets, ICA were detected in only 2 of 30 control sera versus 20 of 21 IDDM sera (Table 2). Antibodies to human islet cells were detected in 20 IDDM patients, using undiluted sera (two cases) or dilutions ranging from 1/4 to 1/32. The fluorescence pattern was identical to that previously described[2] and stained all types of islet cells. These 20 patients included 15 with autoimmune phenomena (cases 6–8, 10–13, 14–17, and 18–21) and all patients with recent-onset diabetes (cases 1–5). In all instances, ICA were complement-fixing IgG. With mouse islets, the immunofluorescent test was positive with undiluted sera in 18 IDDM patients. Among the three sera negative with mouse islets, one was also negative for human islets (case 9).

Among the 20 IDDM sera tested for other organ-specific antibodies, 5 were positive for gastric parietal cell antibodies (cases 8, 11, 18–20) and 6 were positive for antibodies to thyroid microsomes (cases 6–8, 18, 20, and 21).

Anti-insulin antibodies were present in sera from only 8 of the 21 IDDM patients (cases 6, 8, 9, 12, 14, 16, 18, 19). They were absent in all sera from NIDDM patients.

## DISCUSSION

Our results show that sera from a number of IDDM patients suppress IRI release from mouse islets in vitro, in the presence of complement. Immunoglobulin G from these sera induces the same suppressive effect, while globulin-free sera as well as sera absorbed by prior incubation with islets do not. This functional evidence of anti-beta-cell humoral immunity in some IDDM patients was associated with morphologic alteration of islets, supporting the idea that some CF-ICA are cytotoxic against beta-cells. The fact that glucagon secretion from islets was not inhibited in the presence of the IDDM sera tested would suggest that cytotoxic antibodies, in these cases, were specifically directed against beta-cells. IDDM sera were suppressive on normal human islets. These data are consistent with increasing evidence that humoral immunity may contribute to selective beta-cell damage in some form(s) of human diabetes.

Since 1940, when "insulitis" was first noted in pancreatic islets of patients early in the course of IDDM,[16,17] the concept of an immunologic pathogenesis has been supported by several lines of evidence. There is a high incidence of IDDM in patients with autoimmune diseases such as Hashimoto's thyroiditis, Graves' disease, etc.[18] IDDM is correlated with HLA haplotypes that are closely associated with autoimmune diseases.[19] An involvement of cell-mediated immune processes has been suggested on the basis of the migration inhibition test,[20,21] the passive transfer of diabetes to mice by human leucocytes[22] [although this was not confirmed by further works[23–25]], and the specific suppression of IRI release in vitro by circulating lymphocytes from IDDM patients.[10] This cell-mediated immunity may be due to a deficient suppressor-cell activity.[26] There is increasing evidence that humoral immunity may also be involved in islet damage, although ICA detected by immunofluorescence in sera from IDDM patients[1–4] react with cytoplasmic components of A, B, D, and PP islet cells[2,3] so that they are probably not involved in selective beta-cell damage. Conversely,

TABLE 2
Comparison of ICA detected by immunofluorescence, suppression of insulin release by lymphocytes* and sera in vitro, anti-insulin antibodies, and other organ-specific antibodies

| Case no. | ICA | | Suppression of lymphocytes | IRI release (sera) | Anti-insulin antibodies | Organ-specific antibodies |
|---|---|---|---|---|---|---|
| | Human islets | Mouse islets | | | | |
| 1 | +1/16 | + | − | − | − | − |
| 2 | +1/32 | +w | − | − | − | − |
| 3 | +1/32 | + | + | + | − | − |
| 4 | +1/16 | +w | + | − | − | − |
| 5 | +1/32 | + | + | − | − | − |
| 6 | +1/4 | +w | + | + | − | + |
| 7 | +1/10 | + | + | + | + | + |
| 8 | +1/1 | +w | + | − | + | + |
| 9 | − | − | + | − | + | − |
| 10 | +1/10 | +w | + | + | − | − |
| 11 | +1/4 | +w | + | + | − | − |
| 12 | +1/4 | +w | + | − | + | + |
| 13 | +1/4 | +w | + | + | − | − |
| 14 | +1/4 | − | + | − | + | − |
| 15 | +1/4 | +w | + | − | − | − |
| 16 | +1/1 | + | + | + | + | − |
| 17 | +1/4 | +w | + | + | − | − |
| 18 | +1/10 | +w | + | + | + | + |
| 19 | +1/4 | − | + | − | + | + |
| 20 | +1/4 | +w | + | + | − | + |
| 21 | +1/4 | +w | + | + | − | + |

Results are presented as negative (−), positive (+), or weakly positive (+w) tests. Numbers represent the highest serum dilutions in positive tests.

* Results concerning suppression of IRI release by lymphocytes have been published elsewhere.[10]

beta-cell surface antibodies were found in sera from patients with recent-onset IDDM[5] and are more likely to account for the beta-cell injury. The relationship between ICA and cell-surface antibodies remains unknown. Complement-fixing ICA may be more closely related to the clinical onset of IDDM than cytoplasmic ICA.[7] Anti-islet cytotoxicity of CF-ICA has been demonstrated by investigations using the trypan-blue exclusion test[8] and the $^{51}Cr$ release test.[9] In the latter study, using a double-fluorescence technique, beta-cells were shown to be selectively altered. Our data suggest that such morphologic injury is correlated with functional damage: IRI release was selectively suppressed while glucagon release was not.

When studied by immunofluorescence[5] most ICA appear non-species-specific. Our results confirm that the cytotoxic effect of complement-dependent ICA is detectable on mouse islets. In addition, we have shown that it is also detectable on normal human islets. Although these results appear to be preliminary, because of scarcity of human material, they may be of particular importance since one serum with no detectable cytotoxicity against mouse islets suppressed IRI release from human islets. The role of humoral immunity in IDDM may be underestimated when studied with heterologous systems.

Complement-dependent cytotoxicity against beta-cells was detectable in patients with autoimmune manifestations only when diabetes was present. This evidence supports the involvement of specific anti-islet immunity in the pathophysiology of IDDM.

A point requiring further discussion is the higher than normal basal IRI release measured in the presence of some suppressive IDDM sera. Some possible explanations are: the presence of an ongoing cytolytic IRI release in basal medium, a nonspecific stimulation of the remnant beta-cells by some cytolytic byproducts (e.g., amino acids), and the presence of some paradoxically stimulating beta-cell factor(s).[27] This high basal release after incubation with some IDDM sera was not observed in preliminary perfusion experiments.

When islets were perfused for 45 min with basal medium, basal IRI releases from control and test islets were identical but, unlike control islets, further release did not occur for test islets during the stimulatory period (unpublished data). Thus, the high basal IRI release in static incubation was presumably due to some passive leakage of insulin from damaged cells, as supported by another study.[28] Consistent with this hypothesis, no supplementary IRI release occurred during maximal stimulation by 20 mmol/L glucose + theophylline in the presence of IDDM sera + complement.

We have previously shown that circulating lymphocytes from IDDM patients selectively suppressed IRI release from dispersed mouse islet cells in vitro.[10] In the present series, lymphocytes from all patients with cytotoxic sera suppressed IRI release in vitro (Table 2). However, complement-dependent ICA cytotoxicity may well not be the only immunologic mechanism involved in islet damage. ICA were shown to display antibody-dependent cellular cytotoxicity (ADCC) on human insulinoma cells.[29] A possible role of ADCC has also been suggested by the increased number of killer cells in some IDDM patients.[30]

In all patients with CF-ICA cytotoxicity, ICA were detected by immunofluorescence either on human or on mouse islets (Table 2). Conversely, no correlation was found between complement-dependent ICA cytotoxicity and the presence of circulating anti-insulin antibodies (Table 2). This suggests that anti-insulin antibodies are not implicated in the cytotoxic effect of IDDM sera.

The finding of two subgroups of IDDM sera, suppressive and nonsuppressive, is consistent with a current hypothesis of at least two distinct IDDM syndromes. All suppressive sera but one were obtained from patients with autoimmune phenomena, 1–20 yr after the recognized onset of diabetes. Similarly, in such patients, ICA detected by immunofluorescence persist for a long time.[2,3] This suggests a sustained disorder in the immune response, independent of a persisting antigenic stimulation by the remnant beta-cells.[31] One suppressive serum was obtained from a recent-onset diabetic patient without detectable autoimmune manifestations. But this patient, whose mother had thyrotoxicosis, may be of the autoimmune type as defined by Bottazzo.[32] Except for this case, the sera of all recent-onset diabetics free from detectable autoimmune diseases were nonsuppressive. In this form of diabetes, the presence of ICA is transient[1–4] and complement-dependent cytotoxic ICA, if demonstrated, may result from a transient outburst of host defences against, for example, viral infections.[33,34] This outburst may decline quickly when antigenic stimulation stops. In our study, patients were not selected at random. More experiments are thus needed to assess the significance of humoral immunity in the most common form of IDDM (e.g., without detectable autoimmune phenomena).

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**REFERENCES**

[1] Bottazzo, G. F., Doniach, D., and Pouplard, A.: Humoral autoimmunity in diabetes mellitus. Acta Endocrinol. (Copenh.) *83* (Suppl. 205):55–61, 1976.

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

[3] MacCuish, A. C., Barnes, E. Z., Irvine, Z. J., and Duncan, L. J. P.: Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet *2*:1529–33, 1974.

[4] Lendrum, R., Walker, G., Cudworth, A. G., Theophanides, C., Pyke, D. A., Bloom, A., and Gamble, O. R.: Islet cell antibodies in diabetes mellitus. Lancet *2*:1273–76, 1976.

[5] Lernmark, A., Freedman, Z. R., Hofmann, C., Rubenstein, A. H., Steiner, D. F., Jackoon, R. L., Whiter, R. J., and Fraisman, H. S.: Islet cell surface antibodies in juvenile diabetes mellitus. N. Engl. J. Med. *299*:375–80, 1978.

[6] Maclaren, N. K., Huang, S. N., and Fogh, J.: Antibody to cultured human insulinoma cells in insulin-dependent diabetes. Lancet *2*:997–1000, 1975.

[7] Bottazzo, G. F., Dean, B. M., Gorsuck, A. N., Cudworth, A. G., and Doniach, D.: Complement-fixing islet cell antibodies in type 1 diabetes: possible monitors of active beta-cell damage. Lancet *1*:668–72, 1980.

[8] Rittenhouse, H. G., Oxender, D. L., Pek, S., and Ar, D.: Complement mediated effects on pancreatic islets with sera from diabetic patients. Diabetes *29*:317–22, 1980.

[9] Dobersen, M. J., Scharff, J. E., Ginsberg-Fellner, F., and Notkins, A. L.: Cytotoxic autoantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. N. Engl. J. Med. *303*:1493–98, 1980.

[10] Boitard, C., Debray-Sachs, M., Pouplard, A., Assan, R., and Hamburger, J.: Lymphocytes from diabetics suppress insulin release in vitro. Diabetologia *19*:259, 1980.

[11] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35–39, 1967.

[12] Yalow, R. S., and Berson, J. A.: Immunoassay of endogenous insulin in man. J. Clin. Invest. *39*:1157, 1960.

[13] Assan, R., Tchobroustski, G., and Derot, M.: Glucagon radioimmu-

noassay: technical problems and recent data. Horm. Metab. Res. 5 (Suppl. 1):82–90, 1971.

[14] Rosselin, G., Assan, R., Yalow, R. S., and Berson, S. A.: Separation of antibody bound and unbound peptide hormones labelled with iodine 131 by talcum powder and precipitated silica. Nature *212*:355–57, 1966.

[15] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, A., and Newerly, K.: Insulin-I 131 metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin-treated subjects. J. Clin. Invest. *35*:170–90, 1956.

[16] Von Meyenburg, H.: Veber "Insulitis" bei Diabetes. Schweiz Med. Wochenschr. *21*:554–57, 1940.

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

[18] Mac Cuish, A. C., and Irvine, W. J.: Autoimmunological aspects of diabetes mellitus. Clin. Endocrinol. Metab. *4*:435–71, 1975.

[19] Cudworth, A. G.: Type I diabetes mellitus. Diabetologia *14*:281–91, 1978.

[20] 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.

[21] MacCuish, A. G., Jordan, J., Campbell, C. J., Duncan, L. J. F., and Irvine, W. J.: Cell-mediated immunity to human pancreas in diabetes mellitus. Diabetes *23*:693–97, 1974.

[22] Buschard, K., Madsbad, S., and Rygaard, J.: Passive transfer of diabetes mellitus from man to mouse. Lancet *1*:908–10, 1978.

[23] Thurneyseen, O., Jansen, F. K., Vialettes, B., Vague, P., Selam, J. L., and Mirouze, J.: Passive transfer of lymphocytes from diabetic man to athymic mouse. Lancet *1*:1291–92, 1979.

[24] Sitges Sena, A., Farndon, J. R., Shenton, B. K., and Johnston, I. D. A.: Passive transfer of lymphocytes from diabetic man to athymic mouse. Lancet *1*:1292, 1979.

[25] Neufeld, M., McLaughlin, J., Maclaren, W. K., Rosenbloom, E., and Donnelly, W.: Failure to transfer diabetes from man to mouse. N. Engl. J. Med. *301*:665, 1979.

[26] Buschard, K., Madsbad, S., and Rygaard, J.: Depressed suppressor cell activity in patients with newly diagnosed insulin-dependent diabetes mellitus. Clin. Exp. Immunol. *41*:25–32, 1980.

[27] Nielsen, J. H., Eff, C., Deckert, T., and Andersson, A.: Stimulatory effect of serum from diabetic patients on insulin release from mouse pancreatic islets maintained in tissue culture. Diabetologia *20*:60–65, 1981.

[28] Kanatsuna, T., Lernmark, A., Rubenstein, A. H., and Steiner, D. F.: Block in insulin release from column-perifused pancreatic $\beta$-cells induced by islet cell surface antibodies and complement. Diabetes *30*:231–34, 1981.

[29] Huang, S. W., and Maclaren, N. K.: Insulin-dependent diabetes: a disease of autoaggression. Science *192*:64–66, 1976.

[30] Pozzili, P., Sensi, M., Gorsuck A. N., Bottazzo, G. F., and Cudworth, A. G.: Evidence of raised K-cell levels in type I diabetes. Lancet *2*:173, 1979.

[31] Madsbad, S., Bottazzo, G. F., Cudworth, A. G., Dean, B., Faber, U. K., and Binder, C.: Islet-cell antibodies and beta-cell function in IDD. Diabetologia *18*:45–47, 1980.

[32] Bottazzo, G. F., Cudworth, A. G., Moul, D. J., Doniach, D., and Festenstein, H.: Evidence for a primary autoimmune type of diabetes mellitus. Br. Med. J. *2*:1253–55, 1978.

[33] Champsaur, H., Dussaix, E., Samolyk, D., Fabre, M., Bach, C., and Assan, R.: Diabetes and Coxsackie B 5 infection. Lancet *2*:251, 1980.

[34] Yoon, J. W., Austin, M., Onodera, T., and Notkins, A. L.: Virus induced diabetes mellitus: isolation of a virus from the pancreata of a child with diabetic ketoacidosis. N. Engl. J. Med. *300*:1173–79, 1979.