Presence of complement-dependent cytotoxic activity against clonally-derived rat islet tumour cells in sera from Type 1 (insulin-dependent) diabetic patients and control subjects

D. E. Cavender*, M. A. Virji and S. Holze-Joost**

Department of Epidemiology, Graduate School of Public Health and Departments of Pathology and Pediatrics, University of Pittsburgh School of Medicine and the Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA

Summary. Heat-inactivated sera from newly diagnosed Type 1 (insulin-dependent) diabetic patients and control subjects were tested for the presence of antibodies to islet cell surface antigens by means of a sensitive immunofluorescent, micro-cytotoxicity assay using two clones of a rat islet cell tumour as antigens. Complement-dependent cytotoxicity was found in 74% of diabetic patient sera and 87% of control sera, and there were no significant differences in titres between diabetic patients and control subjects. A minority of the sera from both patients and controls were cytotoxic for only one of the two clones, suggesting the presence of multiple antigen-antibody systems. Preadsorptions of the sera with rat liver powder,

sheep erythrocytes, and/or protein A-conjugated agarose beads were inconsistently effective in decreasing levels of lytic activity in control sera. It is concluded that more information is required concerning the antigens of rat islet cells and islet cell cytotoxic factors present in normal sera before such cells and assays can be reliably used for the detection of islet cell surface antibodies.

Key words. Type 1 diabetes, complement-dependent cytotoxicity, immunofluorescent, microcytotoxicity assay, clonal islet-tumour cells.

The involvement of immunologic processes in the pathogenesis of Type 1 (insulin-dependent) diabetes mellitus was first suggested by the observation that pancreatic islets from newly diagnosed Type 1 diabetic patients often contain a large number of infiltrating lymphocytes [1]. Subsequent in vitro studies demonstrated the presence of both humoral [2–4] and cellular [5, 6] immune-reactivity to pancreatic antigens in Type 1 diabetic patients, suggesting possible autoimmune mechanisms in this disease.

Autoantibodies to both cytoplasmic [2, 3] and cell-surface [4] islet cell antigens have been described, and are usually referred to as ICA and ICSA respectively. Both ICA and ICSA appear to be organ specific but not species specific [2, 7]. However, ICA react with all four known types of hormone-producing cells in the islets [8], while ICSA are often B cell-specific [9, 10]. In addition, ICSA-containing sera are often cytotoxic for B cells in the presence of complement [11]. These observations suggest that ICA may be a nonspecific marker

arising as a consequence of islet cell destruction, while ICSA may be a specific diabetogenic factor [12].

Due to the difficulty of obtaining large numbers of viable human islet cells or islets from experimental animals, several laboratories [13–15] have recently used clonal lines [16] of a X-ray-induced rat islet cell tumour [17] as a substrate for the detection of ICSA. These studies have shown that cytotoxic activity directed against the clonally derived cells can be detected in the sera of Type 1 diabetic patients. The question of specificity of the cytotoxic reaction observed has not been fully investigated and remains to be resolved if these cells are to be used extensively. In this communication, we report the results of a study of the specificity of the cytotoxicity reaction of the diabetic sera directed against these clonal cells.

Subjects and methods

Patients and sera

Since February 1979, all newly diagnosed Type 1 diabetic patients less than 17 years of age and seen at the Children's Hospital of Pittsburgh, Penn, USA have been asked to participate in an ongoing consecutive admission study of Type 1 diabetes [20]. The protocol for this study, approved by the institutional review committee, includes HLA typing

Present addresses:

* Department of Internal Medicine, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235, USA

** University Children's Clinic, Medical School, University of Göttingen, Humboldtallee 38, D-3400 Göttingen, FRG

Fig. 1. A-D Immunofluorescent microcytotoxicity assay for ICSA. RIN-m5F (A, a, B, b) and 14B (C, c, D, d) cells grown in Terasaki plate wells were used for the complement-dependent cytotoxicity assay to detect ICSA as described in the text. The cells were photographed in situ in the wells; since the wells are conical, the plane of focus is uneven. The cells are shown under polarizing (A-D) or fluorescent (a-d) light. A, a and C, c are for the same patient serum ($\times 95$ magnification), with 90% dead cells. B, b and D, d are for the same control serum ($\times 190$ magnification), with 90% and 25% dead cells respectively

and autoantibody testing as well as the collection of other genetic, immunologic and epidemiologic data. Serum samples from 39 of the most recently diagnosed patients were used in the study reported here.

Control serum samples included 19 randomly selected specimens obtained from normal non-diabetic individuals sent to our clinical laboratory for various analyses, and 5 samples from normal laboratory personnel who were not animal handlers and did not work in laboratories where animals were used. All sera were kept frozen at -20°C in small aliquots until use. Just prior to testing all sera were heat-inactivated at 56°C for 30 min, and were then spun for 10 min at 100,000 rpm on an AirfugeTM ultracentrifuge (Beckman Instruments, Palo Alto, Calif., USA) to remove possible immune complexes.

Insulinoma cells and culture

The RIN-m5F and RIN-m14B (hereafter referred to as 5F and 14B) clones [16] of a transplantable rat islet tumour [17] were kindly provided by Drs. H. Oie and A. Gazdar. The 5F and 14B clones secrete primarily insulin and somatostatin respectively [21]. Both lines were routinely grown in 25 cm² tissue culture flasks (Falcon, Oxnard, Calif., USA) in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin). The cells have a doubling time of 20 h (M.A. Virji and M.-J. Dimasi, unpublished observations). At the time of passage, the cells were counted, resuspended at 10^5 cells/ml in fresh medium and $10\ \mu\text{l}$ plated per

well in Terasaki plates (Falcon, Oxnard, Calif., USA). After 48–96 h of incubation at 37°C in a humidified atmosphere of 5% CO₂ in air, the cells were washed by flooding the plate with warm culture medium, pouring off the excess and tapping the plate sharply on a paper towel. The adherent cells were used as the target antigen for the ICSA assay described below.

The 5F and 14B cells were used in the ICSA assays between passage numbers 32 and 49 and between passage numbers 41 and 73 respectively. The cells were periodically tested for the presence of mycoplasma and kept free of microbial contamination. The cells secreted insulin and somatostatin respectively. 5F cells did not respond to glucose stimulation, but glyceraldehyde (15 mmol/l) caused a 1.5- to 2-fold increase in insulin release during a 1-h stimulatory period when tested during different passage numbers. The 14B cells maintained a low level of somatostatin secretion which did not change from passage to passage.

Assays for islet-cell cytoplasmic antibodies (ICA) and islet cell surface antibodies (ICSA)

ICA were detected by an immunohistochemical procedure using frozen cryostat sections of monkey pancreas, 1:2 dilution of serum in phosphate-buffered saline (PBS) and glucose-oxidase conjugated second antibody as described previously [22, 23].

ICSA were detected by a complement-dependent immunofluorescent microcytotoxicity assay as follows. Duplicate 2 µl aliquots of doubling dilutions of sera (1:2, 1:4, 1:8) in culture medium were added to Terasaki plate wells containing washed, adherent confluent monolayers of 5F or 14B cells and the plates were reincubated for 30 min at 37°C in an atmosphere of 5% CO₂ in air. The plates were then washed twice as described above. Next, 5 µl of an optimal dilution (1:4) of a single prescreened lot of rabbit serum as the source of complement (see below), in culture medium, were added per well and incubated for 60 min as described above. Two ul of a 1% solution of ethidium bromide (w/v) in PBS, pH 7.2 with 5% EDTA (w/v) were then added, and the plates were examined by a combination of fluorescence/phase microscopy using a Leitz Dialux 20 microscope (Leitz, Wetzlar, FRG). Orange-red optics were used for the fluorescence. The percentage of dead cells was estimated as ≤10% (negative for cytotoxicity), 25%, 50% 75%, or ≥90% (positive) (Fig. 1). A hyper-immune rabbit anti-rat islet cell serum, kindly donated by Dr. M.J. Dobersen [11], was used as a positive control in all experiments. This serum always killed ≥90% of both the 5F and 14B cells at a 1:32 dilution. A prescreened lot of pooled human AB sera (Medical Specialties Laboratory, Chicago, Ill., USA) was used as a negative control in each assay. Its toxicity was always less than 10% in the presence of complement.

The rabbit serum as the complement source was titrated against the positive control serum and four ICSA+ sera from diabetic patients in a checkerboard fashion. The rabbit serum was noncytotoxic by itself at a 1:2 dilution and gave equivalent cytotoxicities at 1:2 and 1:4 dilutions after incubation of the insulinoma cells with ICSA+ sera at various dilutions. Therefore, a 1:4 dilution of the rabbit serum was used throughout these studies.

Adsorption of sera

To remove possible antibodies with reactivity to Forssman-like antigens or non-islet rat antigens, undiluted sera were adsorbed with various numbers of sheep erythrocytes (SRBC, Colorado Serum Company, Denver, Colo., USA) or various amounts of acetone-extracted rat liver powder (RLP, Sigma Chemical Company, St. Louis, Mo., USA), respectively, for different lengths of time and at different temperatures, as noted in the text. SRBC or RLP were then removed by centrifugation (500 g, 5 min).

Adsorption of sera with protein A-conjugated agarose beads (E-Y Laboratories Inc., San Mateo, Calif., USA) was carried out at 4°C in a cold room. Each millilitre of the suspended beads contains 2 mg of protein A with a binding capacity of 20 mg for human IgG. One-half ml of beads was withdrawn from the bottle, washed once in RPMI-

1640, and pelleted by centrifugation (500 g, 5 min). One-half ml of undiluted serum was then mixed thoroughly with the pellet and incubated on ice for 5 min. The beads were then removed by centrifugation. Immunoglobulin levels and ICSA activity in the supernatants were then compared to those in the unadsorbed sera.

In a few cases, the pelleted beads were washed three times in PBS, resuspended in 0.5 ml of 0.2 mol/l glycine-hydrochloric acid buffer (pH 2.5), and incubated on ice for 5 min. Following a final centrifugation, the eluted material from the beads was neutralized with 15 µl of a 7.5% sodium hydroxide solution (w/v) and dialyzed against a large volume of PBS overnight in the cold room. The dialyzed material was then directly tested for ICSA as described above.

Statistical analysis

The cytotoxicity reactions of the control and diabetic sera against the tumour cells were compared by using chi-square analysis. Significance for differences between reactivity was set at $p = 0.05$ or less.

Results

Prevalence of ICSA in sera from Type 1 diabetic patients and control subjects

Sera from 75% (29/39) of the Type 1 diabetic patients and 87% (21/24) of the control subjects had ICSA activity when using >10% cell death as the definition of ICSA-positivity (Table 1). The data in Table 2 show that the percentages of ICSA+ sera among patients and controls were not significantly different ($p > 0.05$) regardless of the percentage of cell death that was used to define a serum as positive or negative. Ultracentrifugation of the sera to remove immune complexes following heat inactivation had no effect on the results (data not shown). The majority of the positive sera for the patient and control groups had reactivity towards both the insulin-secreting 5F line and the somatostatin-secreting

Table 1. ICSA activity in sera from Type 1 diabetic patients and control subjects against rat islet tumour-derived cell lines

Group	Positive ^a			Negative Neither line	Total
	Both 5F and 14B	5F only	14B only		
Type 1 diabetic patients	20	5	4	10	39
Control subjects	17	4	0	3	24

^a >10% cytotoxicity at 1:2 dilution; 5F = RIN-m5F (insulin producing); 14B = RIN-m14B (somatostatin producing)

Table 2. Sera positive for ICSA using different cell lysis cut-off points

% of cell lysis used to define ICSA-positivity	5F cells (%)		14B cells (%)	
	Diabetic patients (n = 39)	Control subjects (n = 24)	Diabetic patients (n = 39)	Control subjects (n = 24)
> 10	64	88	62	71
> 25	54	75	54	58
> 50	44	46	36	42
> 75	23	38	23	29

Table 3. Serum ICSA titres against 5F and 14B cell lines in diabetic patients and control subjects

Group	No. ICSA positive sera tested ^a	5F cells			14B cells		
		≤1:2	1:4	≥1:8	≤1:2	1:4	≥1:8
Type 1 diabetic patients	26	11	4	11	16	5	5
Control subjects	21	8	7	6	10	5	6

^a The 47 sera tested here included 26 of the 29 ICSA+ sera from Type 1 diabetic patients and all 21 ICSA+ sera from control subjects noted in Table 1. Three of the 29 ICSA+ diabetic patients from Table 1 did not have enough sera available to determine titres; the sera from all 3 patients killed ≥25% of both 5F and 14B cells at a 1:2 dilution

Table 4. Cytotoxic activity in adsorbed and unadsorbed control sera^a for 5F and 14B cells

Adsorption protocol ^a	Serum sample											
	1		2		3		4		5		6	
	5F	14B	5F	14B	5F	14B	5F	14B	5F	14B	5F	14B
Unadsorbed	75	>90	75	75	>90	25	>90	100	50	50	50	50
10 mg/ml RLP, 4°, 16 h	75	>90	25	50	>90	10	>90	100	25	50	50	50
10 mg/ml RLP, 37°, 30 min 4 ×	75	>90	10	25	75	10	>90	100	25	25	50	25
40 mg/ml RLP, RT, 60 min	NT	NT	25	50	NT	NT	>90	100	<10	25	50	50
5 × 10 ⁸ SRBC/ml, 37°, 30 min 4 ×	NT	NT	<10	50	NT	NT	>90	100	<10	50	50	25
5 × 10 ⁸ SRBC/ml, 37°, 30 min 2 × + 10 mg/ml RLP, 37°, 30 min 2 ×	50	>90	NT	NT	50	10	>90	100	NT	NT	NT	NT

^a 1:2 dilution of serum; RLP=rat liver powder; NT=not tested; SRBC=sheep erythrocytes; RT=room temperature; 2 × /4 × = two/four consecutive adsorptions following centrifugation

14B line. Nine of the 13 sera which killed only one of the two cell lines were cytotoxic for the 5F line. Retesting of these 13 sera an average of three times each gave the same result. All five sera from normal healthy laboratory control subjects and 12 sera from the other control subjects were cytotoxic for both the cell lines.

Dependence of ICSA activity on the presence of complement

To determine whether complement activation was necessary for the cytotoxic reactions observed, two types of experiments were performed. Substitution of medium for rabbit serum in the second stage of the ICSA assay abolished all cytotoxicity. In addition, none of the sera was cytotoxic if heat-inactivated rabbit serum (56°, 30 min) was used in place of untreated rabbit serum.

Titres of ICSA in patient and control sera

An artificial categorization of sera according to titre levels as low (≤1:2), intermediate (1:4) or high (≥1:8) showed that 20% to 45% of both patient and control sera had high ICSA titres against both the 5F and 14B lines (Table 3). There was a greater proportion of diabetic patients than control subjects with high titres against the 5F line and a lower proportion with high titres to the 14B line, but neither of these two differences was statistically significant. Though high ICSA titres

Fig. 2. Correlation of ICSA titres against 5F and 14B cell lines for diabetic patients (●) and control subjects (○). The ICSA titres were determined by the ethidium bromide immunofluorescence, microcytotoxicity assay. The sera were diluted with the culture medium RPMI 1640. The cytotoxicity for the same sera against 5F and 14B cells was determined in parallel assays

against both the cell lines were observed in many sera, there was almost an equal number of samples in which the titres against the two cell lines did not show a correlation (Fig. 2).

Adsorption studies

Based on previous reports [7, 11, 13], several adsorption protocols using rat liver powder (RLP) and/or sheep erythrocytes (SRBC) were tested for their effects on

Fig. 3. Effect of immunoadsorption with protein A-agarose on ICSA titres in control sera. The cytotoxicity activity in 5 control sera against 5F cells was assayed by the immunofluorescence, microcytotoxicity assay, before (A) and after (B) adsorption with protein A-conjugated agarose beads; and also in the neutralized eluates (C) from the beads. Four serum samples were from the diabetic patients in Table 4: Patient 4 (○); 2 (△); 5 (□); 6 (▲). One sample was from a patient (●) not shown in Table 4

ICSA activity in sera from control subjects. The results shown in Table 4 demonstrate that no adsorption procedure was consistently effective in removing (or even lowering) ICSA activity. Furthermore, in a few cases ICSA reactivity was removed for just one of the two lines. Similarly, we were unable to reduce to background levels ($\leq 10\%$) the cytotoxicity found in five ICSA+ control sera by adsorption on monolayer cultures of rat embryo fibroblasts (data not shown).

Adsorption of five control sera, which showed high cytotoxic activity against 5F cells, with protein A-conjugated agarose beads, gave the results shown in Figure 3. Adsorption was successful in removing ICSA from two of the five sera and the ICSA activity was recovered in the neutralized eluate from the beads. Adsorption of the other three sera with the beads had no marked effect on their ICSA levels, even though the IgG concentrations were reduced by approximately 90%. Additionally, neutralized eluates from the protein A-conjugated beads used to adsorb these three sera had no demonstrable ICSA activity.

Correlation between ICA and ICSA activity in patient and control sera

Twenty-eight of the sera from 38 diabetic patients, but none of the 24 control sera, were positive for islet cell cytoplasmic antibodies (ICA) as identified by using frozen cryostat sections of monkey pancreas. There was no correlation between the presence of ICA and either the presence or titres of ICSA in the patient group (data not shown).

Discussion

The results presented here demonstrate that sera from most individuals, whether Type 1 diabetic patients or

control subjects, contain cytotoxic factors for cultured rat islet tumour cells. In all cases, the cytotoxic reaction was dependent on the presence of both heat-inactivated human serum and untreated rabbit serum, suggesting that most human sera contain antibodies capable of both binding to transformed rat islet cells and fixing rabbit complement. Despite this universal dependence on rabbit serum, marked differences between the human sera were observed in other characteristics. First, ICSA activity was inconsistently removed from control sera by non-specific adsorptions with sheep erythrocytes and/or rat liver powder (Table 4). Secondly, there was a wide variation in the titres of cytotoxic activity (although diabetic patients and control subjects did not differ in this regard, Table 3). Thirdly, individual sera often had markedly different titres for the two cell lines used or sometimes were even cytotoxic for only one of the lines (Fig. 2); for example, one serum from a patient killed 50% of the 5F cells at a 1:8 dilution but was completely negative on 14B cells, even at a 1:2 dilution. Finally, adsorption of ICSA-positive sera with protein A-conjugated agarose beads gave variable results (Fig. 3).

Two previous studies from other laboratories have suggested that sera from Type 1 diabetic patients are significantly more cytotoxic against 5F cells (as measured by a ^{51}Cr release assay) than are sera from control subjects [13, 14]. However, their data also show that there is considerable overlap in the distributions of cytotoxic activities between sera from diabetic patients and control subjects, and that it is only in the extreme case of very marked cytotoxicity that an individual serum could be definitely classified as coming from a diabetic patient. In addition, although Eisenbarth et al. (1981) found a significant correlation between the levels of ^{51}Cr release where sera were simultaneously tested against the 5F and 14B clones, they also appeared to find some sera which were markedly more cytotoxic against one of the two clones [13]. It has also been reported that adsorption of ICSA positive sera with anti-immunoglobulin reagents gives variable results [13]. That these observations may not be peculiar to transformed rat islet cells only is indicated by our preliminary observations on cytotoxic activity in a smaller group of samples from control subjects and Type 1 diabetic patients (3 and 12 respectively) in which ^{51}Cr -labeled normal rat islet cells were used as the antigen source. In the presence of rabbit complement alone, the label release was $4 \pm 1.5\%$ (mean $\pm$ SD, $n = 10$). However, in the presence of control sera the label release was 20.5 ± 5.9 ; for the diabetic sera, it was 37.1 ± 6.8 with a marked overlap between the patient and control sera. Even though the patients tended to have higher values, it was difficult to assign a cut-off point above which cytotoxic activity would definitely be indicative of the presence of autoantibodies.

Finally, it should be noted that the development of similar fluorescent, microcytotoxicity assays have been independently described in two other laboratories [25,

26]. In the first case, normal mouse islet cells isolated by dispase treatment were used as the antigen source, while recently trypsinized 5F cells were used in the other study. It was reported in both of these studies that approximately 50% of newly diagnosed Type 1 diabetic patients were ICSA+ compared to almost none of the controls [25, 26]. However, a serum was considered positive in these two reports only if 50% or more of the cells were observed to take up the dye, suggesting that diabetic patients simply had higher titres of cytotoxic activity than the control subjects. This may also have had an effect on the number of patients found positive for ICSA, which was only 45%, indicating the possibility of a large number of false negatives because of the high cut-off point to denote positivity of a serum sample for ICSA [26]. The high cut-off presumably was set to minimize the overlap between cytotoxicity of normal and diabetic sera. In the present study, no definite cut-off point could be established to clearly separate the diabetic patients and control subjects (Table 2). In view of our almost complete lack of knowledge concerning the nature of the relevant islet cell antigens in the etiology of Type 1 diabetes, the use of cells in ICSA assays which have been recently treated with proteases could lead to spurious results due to the possible proteolytic digestion of antigens from the cell surface. Eisenbarth et al. [13], cultured 5F cells overnight following trypsin treatment, which presumably would allow for the re-expression of such antigens; the same method was used in the present study. This is of importance since trypsinization may have an effect on the surface membrane antigens, with proteolytic alterations of the membrane antigens to which control and diabetic sera may react differently and unpredictably. In a study by Toguchi et al [26], cells treated with trypsin just before analysis were used in the assay, introducing a variable with potential effect on cell membrane antigens. Additional uncontrolled variables in that study included incubations at room temperature in unbuffered medium presumably without CO₂, resulting in possible effects on pH maintenance. In the study presented here all these factors were controlled to maintain uniformity of test conditions and assure optimal in vitro cellular environment. Also in contrast to the study of Toguchi et al. [26], the reactivities of both the unadsorbed and extensively adsorbed sera were studied in this investigation in order to understand the nature of complement dependent cytotoxicity against the tumour cells detected in the normal sera.

Our conclusion, based on these studies, is that none of the assays described to date using transformed animal islet cells adequately measures ICSA activity. This conclusion is supported by the observation of a marked overlap in ICSA activity between the diabetic patient and control sera, and from the data which show that ICSA-like activity is often not removed by adsorption with either non-islet animal tissue or anti-immunoglobulin reagents. The discrepant results using the 5F and 14B clones suggest the presence of multiple antigen-an-

tibody systems, further complicating the interpretation of these assays. It has also been suggested that the ability of rodent islet cells to take up ⁸⁶Rb can be inhibited irreversibly by heterologous sera, presumably due to the activation of the alternative complement pathway [27]. Our group has previously reported that use of 5F cells as antigen in an ICA assay results in an unacceptable number of both false positive and false negative reactions [22]. Until it is demonstrated that transformed rodent islet cells possess antigens similar or identical to those to which Type 1 diabetic patients react on human islet cells, and until more information is available on such heterologous systems, considerable caution is necessary when interpreting the results of experiments using animal islet tissue to identify islet cell cytotoxic activity in diabetic patients. Direct comparisons of human and normal rodent tissues for the study of islet cell-surface antigens using sera from Type 1 diabetic patients have led to the same conclusion [18, 19]. Thus, continually growing clonally-derived rat islet tumour cells, though convenient as a source of hormone-producing cells, may not be appropriate for use in detection of ICA and ICSA.

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References

- Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14: 619-633
- Bottazzo GF, Florin-Chistensen F, Doniach D (1974) Islet-cell antibodies in diabetes mellitus with polyendocrine deficiencies. *Lancet* 2: 1279-1283
- MacCuish AC, Barnes EW, Irvine WJ, Duncan LJ (1974) Antibodies to pancreatic islet-cells in insulin-dependent diabetic with co-existent autoimmune disease. *Lancet* 2: 1529-1531
- Maclaren NK, Huang S, Fogh J (1975) Antibody to cultured human insulinoma cells in insulin-dependent diabetics. *Lancet* 1: 997-999
- Neuy J, Andersen OO, Bendixen G, Egeberg J, Poulsen JE (1971) Antipancreatic cellular hypersensitivity in diabetes mellitus. *Diabetes* 20: 693-697
- MacCuish AC, Jordan J, Campbell CJ, Duncan LJ, Irvin WJ (1974) Cell-mediated immunity to human pancreas in diabetes mellitus. *Diabetes* 23: 693-697
- Lernmark A, Freedman ZR, Hofmann C, Rubenstein AH, Steiner DF, Jackson RL, Winter RJ, and Traisman HS (1978) Islet-cell-surface antibodies in juvenile diabetes mellitus. *N Engl J Med* 229: 375-380
- Bottazzo FF, Doniach D (1978) Islet-cell antibodies in diabetes mellitus. Evidence of an autoantigen common to all cells in the islet of Langerhans. *Ric Clin Lab* 8: 29-38
- Dobersen MJ, Scharff JE (1982) Preferential lysis of pancreatic B-cells by islet cell surface antibodies. *Diabetes* 31: 459-462
- Van de Winkel M, Smets G, Gepts W, Pipeleers D (1982) Islet cell surface antibodies from insulin-dependent diabetics bind specifically to pancreatic B cells. *J Clin Invest* 70: 41-49
- Dobersen MJ, Scharff JE, Ginsberg-Fellner F, Notkins AL (1980) Cytotoxic autoantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. *N Engl J Med* 303: 1493-1498

12. Lernmark A, Baekkeskov S (1981) Islet cell-antibodies-theoretical and practical implications. *Diabetologia* 21: 431-435
13. Eisenbarth GS, Morris MA, Searce RM (1981) Cytotoxic antibodies to cloned rat islet cells in serum of patients with diabetes mellitus. *J Clin Invest* 67: 403-408
14. Rabinovitch A, MacKay P, Ludvigsson J, and Lernmark A (1984) A prospective analysis of islet-cell cytotoxic antibodies in insulin-dependent diabetic children. Transient effects of plasmapheresis. *Diabetes* 33: 224-228
15. Dyrber J, Poussier P, Nakhooda F, Marliss EB, Lernmark A (1984) Islet cell surface and lymphocyte antibodies often precede the spontaneous diabetes in the BB rat. *Diabetologia* 26: 159-165
16. Gazdar AF, Chick WL, Oie HK, Sims HL, King DL, Weir GC, Lauris V (1980) Continuous, clonal, insulin- and somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. *Proc Natl Acad Sci USA* 77: 3519-3523
17. Chick WL, Warren S, Chute RN, Like AA, Lauris V, Kitchen KC (1977) A transplantable insulinoma in the rat. *Proc Natl Acad Sci USA* 74: 628-632
18. Baekkeskov S, Nielsen JH, Marner B, Bilde J, Ludvigsson J, Lernmark A (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 298: 167-169
19. Baekkeskov S, Lernmark A (1982) Rodent islet cell antigens recognized by antibodies in sera from diabetic patients. *Acta Biol Med Germ* 41: 1111-1115
20. Eberhardt M (1984) An epidemiologic perspective of the factors associated with the presentation of insulin-dependent diabetes mellitus. Dissertation. University of Pittsburgh, Pittsburgh, Pennsylvania
21. Bhathena SJ, Oie HK, Gazdar AF, Voyles NR, Wilkins SD, Recant L (1982) Insulin, glucagon, and somatostatin receptors on cultured cells and clones from rat islet cell tumor. *Diabetes* 31: 521-531
22. Krell J, Rabin BS (1984) Comparison of an immunohistochemical and immunofluorescence procedure to detect antibody to pancreatic islet cells. *Diabetes* 33: 709-711
23. Cavender D, Rabin B, Finegold D (1984) A new sensitive immunohistochemical assay for islet cell cytoplasmic antibodies (ICA). *Diabetes* 33 (Suppl 1): 63 A (Abstract)
24. Dobersen MJ, Scharff JE, Notkins AL (1980) Microculture system for studying monolayers of functional β -cells. *Endocrinology* 160: 1070-1073
25. Vexiau P, Deschamps I, Jansen F, Homberg JC, and Horo J (1984) Detection of islet cell antibodies by a microcytotoxicity method. *J Immunol Methods* 68: 177-183
26. Toguchi Y, Ginsberg-Fellner F, Rubenstein P (1985) Cytotoxic islet cell surface antibodies (ICSA) in patients with Type I diabetes and their first degree relatives. *Diabetes* 34: 855-860
27. Idahl L-A, Sehlin J, Taljedal I-B, Thornell L-E (1980) Cytotoxic activation of complement by mouse pancreatic islet cells. *Diabetes* 29: 636-642

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Dr. Mohamed A. Virji
Department of Pathology
University of Pittsburgh
School of Medicine
One Children's Place
Main Tower, Room 5845
Pittsburgh, PA 15213
USA