Immune Islet Killing Mechanisms Associated with Insulin-Dependent Diabetes: Three Rabbit Antibody-Mediated Islet Cell Cytotoxicity Models

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Summary. Antibody-mediated islet cell killing mechanisms have been associated with human insulin-dependent diabetes. Several types of antibody-mediated cytotoxic mechanisms exist, but only complement-dependent antibody-mediated cytotoxicity is reported for islet killing. To evaluate further islet cytotoxic antibody mechanisms, we have studied antibody-dependent cellular cytotoxicity and complement augmented antibody-dependent cellular cytotoxicity using polyclonal rabbit anti-rat islet cell immune serum and ⁵¹Cr-labelled dispersed normal rat islet target cells. Maximal immune serum-mediated islet cell specific cytotoxicity was 80% for complement-dependent antibody-mediated cytotoxicity, 40% for antibody-dependent cellular cytotoxicity and 20% for complement-augmented antibody-dependent cellular cytotoxicity. The minimum serum dilution for maximal islet cell cytotoxicity was 1:100 for complement-dependent antibody-mediated cytotoxicity, 1:10 for antibody-dependent cellular cytotoxicity and 1:1000 for complement-augmented antibody-dependent cellular cytotoxicity. These data indicate a

unique optimal serum dilution for each of the three antibody killing mechanisms. Immune serum-mediated cytotoxicity was more specific for rat islet target cells than macrophage target cells. That antibody mediated these cytotoxic events was documented using immune serum-derived, DEAE purified immunoglobulin G which induced killing in all three antibody assays. Both antibody-dependent cellular cytotoxicity assays appear useful for studies of human diabetes, since human non-T mononuclear cells are cytotoxic to islet cells. These results suggest that for studies of potential islet cell killing mechanisms in insulin-dependent diabetes, specific xenogeneic assays exist not only for complement-dependent antibody-mediated islet cytotoxicity, but also for antibody-dependent cellular cytotoxicity and complement augmented antibody-dependent cellular cytotoxicity.

Key words: Islet cell cytotoxicity, complement, antibodies, diabetes.

Immune mechanisms have been implicated in the pathogenesis of Type 1 (insulin-dependent) diabetes [1, 2]. Most reports are of indirect observations, but recent studies have attempted to associate immune mechanisms to human diabetes using complement-dependent antibody-mediated cytotoxicity (C'AMC) assays [3–7]. Cytotoxicity assays *in vitro* and the reported results vary, but each method described uses islet derived target cells. One report suggests that virtually all sera from Type 1 diabetic patients have C'AMC against cultured rat islet cells, and that sera from non-diabetic parents and siblings also mediated C'AMC [6]. Using intact normal rodent islets or rat or human islet tumour target cells, 10%–30% of patients' sera have C'AMC [3–5, 7]. Other potential antibody-mediated cytotoxic mechanisms in diabetes have not been examined in detail.

We have developed three relatively simple and reproducible islet specific cytotoxicity assays using non-cultured, non-tumour, normal dispersed rat islet target

cells. In the studies reported here, a polyclonal rabbit anti-rat islet cell antibody model is used in conjunction with rat islet target cells and human non-T mononuclear effector cells. This model is used to evaluate not only complement-dependent antibody-mediated cytotoxicity (C'AMC), but also antibody-dependent cellular cytotoxicity (ADCC), and complement-augmented antibody-dependent cellular cytotoxicity (C'ADCC). We have found the application of these assays useful in studies of the pathogenesis of human insulin-dependent diabetes as previously described [8].

Subjects and Methods

Subjects

Five normal subjects (two women and three men) aged 18 to 32 years understood and signed consent forms. All had normal fasting blood glucose concentrations and none had a family history of diabetes.

Fig. 1. Time course of ⁵¹Cr uptake by dispersed rat islet cells. ⁵¹Cr (50 μ Ci) was incubated with 10^5 islet cells. At 30, 60, 90 min, 2×10^4 cells were removed, washed and the radioactivity estimated. Each data point represents the mean $\pm$ SEM of four separate experiments, using separate target cell preparations on separate days

Fig. 2. Spontaneous ⁵¹Cr release of dispersed rat islet cells. ⁵¹Cr-labelled islet cells were incubated, centrifuged and the radioactivity estimated from the supernatants at the times indicated. Data shown are from $n=5$ experiments at 1, 2, 6 and 18 h, $n=28$ at 4 h and $n=36$ at 8 h (mean $\pm$ SEM). Each n reflects experiments performed on separate days using separate target cells

Mononuclear Cell Preparation

Peripheral venous blood from subjects was mixed with 20 units/ml of preservative-free heparin (Sigma Chemicals, St. Louis, Missouri). Mononuclear cells were isolated using Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, New Jersey) as described by Böyum [9]. Cells harvested from the interface were washed three times in Ca- and Mg-free Hank's balanced salt solution (Grand Island Biochemical Company [GIBCO], Grand Island, New York). Cells (4×10^6 cells/ml) were resuspended in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2.5 mmol/l HEPES buffer (GIBCO), 100 U/ml penicillin and 100 μ g/ml streptomycin (GIBCO). Non-T mononuclear cells were enriched by depletion of T cells using 16-h E-rosetting. Rosette-forming cells were separated using Ficoll-Hypaque centrifugation. Non-T mononuclear cells obtained from the interface were washed three times and resuspended in the incubation medium.

Target Cell Preparation

Inbred Wistar-Furth (RT1^u) or Lewis (RT1^l) rat islets were isolated using collagenase and purified by centrifugation and visual selection as

described previously [10]. Lymph node contamination was avoided by using tangential white light and a black background during visual selection. Target cells were prepared by a modification of a technique described previously [11]. In brief, purified intact islets were incubated at 37°C for 1–2 days in Dulbecco's Modified Eagles Medium (DMEM) and 10% fetal calf serum under 5% CO₂ and 95% humidified air. Individual intact islets were visually removed (no acinar debris or lymph nodes present) and dispersed into a single cell suspension by incubation in 200 μ g/ml DNAase I (Sigma) in Swim's low calcium medium (GIBCO) containing 1 mmol/l EGTA (Sigma) and 1% bovine serum albumin (Miles Laboratories, Kankakee Illinois). The dispersed cells ($2-3 \times 10^6$) were washed, placed in 250 μ l DMEM with 10% fetal calf serum and incubated with ⁵¹Cr (50 μ l sodium chromate, 1 mCi/ml, New England Nuclear, Cambridge, Massachusetts) at 37°C for 30 min. After washing, the Cr-labelled islet target cells, contained 2500–4000 cpm per 10^4 cells and >90% viability was shown by Trypan blue exclusion. Mean $\pm$ SEM islet target cell spontaneous ⁵¹Cr release was $16 \pm 0.4\%$ ($n=28$) at 4 h and 21 ± 0.6 ($n=36$) at 8 h, where n includes experiments performed on separate days using separately prepared effector and target cells. Wistar-Furth peritoneal macrophage target cells were prepared by repeated collections of peritoneal washings from normal animals. These cells were incubated with 100 μ l ⁵¹Cr for 2 h at 37°C and after washing, they contained approximately 2000 cpm/ 10^4 peritoneal cells. Spontaneous ⁵¹Cr release was $19 \pm 2\%$ ($n=3$) at 4 h and $35 \pm 3\%$ ($n=3$) at 8 h, n representing separate experiments as described above.

Rabbit Anti-Rat Islet Cell Antisera and Antibody Preparation

Wistar-Furth dispersed islet cells (10^6) were injected into popliteal lymph nodes of anaesthetized New Zealand rabbits and complete Freund's adjuvant used as described previously [12]. Booster intravenous injections of 10^6 islet cells were administered at 2-week intervals. Adsorption studies were performed using rabbit serum (1 ml) incubated with 3×10^7 Wistar Furth spleen cells for 1 h at room temperature followed by overnight adsorption at 4°C [13]. Immune and normal rabbit IgG was isolated from serum using ammonium sulphate fractionation and DEAE-cellulose anion exchange chromatography as described previously [14].

Cytotoxicity Assays

Triplicate tubes contained DMEM, 10% fetal calf serum and 10^4 serum- or antibody-coated islet target cells and, in all but the C'AMC assay, variable quantities of effector cells. Incubations were from 1–8 h at 37°C under 5% CO₂ and 95% humidified air. ⁵¹Cr-labelled target cells were coated by incubating various dilutions of heat inactivated rabbit anti-rat islet cell serum, normal rabbit serum or purified immune or normal IgG preparations with $1-5 \times 10^5$ islet target cells at 37°C for 30 min in 1.8 ml polypropylene tubes. Excess serum or IgG was removed by centrifugation at $11,600 \times g$ for 15 s and target cells were washed twice with 1 ml DMEM containing 10% fetal calf serum. For C'AMC, target cells were coated with heat-inactivated serum or IgG and incubated with 5% (final concentration) fresh normal rabbit serum (exogenous complement source). Similarly prepared tubes containing heat-inactivated 5% normal rabbit serum (complement-inactivated) were used for spontaneous ⁵¹Cr release estimates. For cell-mediated cytotoxicity, non-T mononuclear cells from normal control subjects were used, and ⁵¹Cr spontaneous release was determined from tubes without added mononuclear cells. For ADCC, non-T mononuclear cells from normal subjects were added to incubation tubes containing heat-inactivated immune or normal rabbit serum or purified immune or normal IgG. Spontaneous ⁵¹Cr release for ADCC was determined from tubes containing rabbit anti-rat islet cell serum-coated islet target cells but no mononuclear cells. For C'ADCC assays, procedures were as described for ADCC except that experimental tubes contained 5% fresh normal rabbit serum as a source of complement, and spontaneous ⁵¹Cr release estimates were derived from tubes identical to the C'AMC assay which permitted correction for complement-dependent antibody-induced lysis (see below). Be-

Fig. 3. Complement-dependent antibody-mediated cytotoxicity (C'AMC) for rabbit anti-rat islet cell serum-mediated C'AMC (●—●) and normal rabbit serum (○—○). Data points (mean $\pm$ SEM) are for $n=12$ at dilutions 10, 10², and 10³ and $n=8$ at dilutions 0.5, 1, 20 and 40×10^4 of rabbit anti-rat islet cell serum. For normal rabbit serum $n=8$ for each data point. (See Fig. 2 for definition of n). Values at dilutions 5×10^3 were not consistently different from those of control normal rabbit serum mediated C'AMC

Fig. 4. Complement-dependent antibody-mediated cytotoxicity (C'AMC) time course and specificity. Specific ⁵¹Cr release by Wistar-Furth rat islet target cells (●—●), Lewis rat islet target cells (■) and Wistar-Furth macrophage target cells (□), using rabbit anti-rat islet cell serum at 1:100 dilution. Data represent (mean $\pm$ SEM) $n=3$ for Wistar-Furth islet cells at 1, 2 and 6 h, $n=6$ for 4 and 8 h, and for Lewis islet target cells $n=12$ at 6 for 4 and 8 h, respectively. For macrophage target cells $n=3$. (see Fig. 2 for definition of n)

fore and after incubations for C'AMC, cell mediated cytotoxicity, ADCC or C'ADCC, the tubes were mixed, centrifuged at $11,600 \times g$ for 15 s or 2 min, respectively. Supernatants and pellets were assessed for radioactivity. Calculations were based on cytotoxicity equivalent to experimental ⁵¹Cr released minus spontaneous ⁵¹Cr released divided by maximal ⁵¹Cr released (Triton X) minus spontaneous ⁵¹Cr released [15]. Triton X releases 97%–100% of ⁵¹Cr bound by islet cells and 88%–92% bound by macrophage cells. For ADCC and C'ADCC assays, cell mediated cytotoxicity was subtracted from the apparent ADCC and C'ADCC values calculated as described above. Cell mediated cytotoxicity at 4 h incubations ranges from 12%–21% specific cytotoxicity. These calculations corrected for cytotoxicity related to direct cell-mediated killing. Thus, the effects of immune sera, IgG, complement and effector cells are each corrected prior to the final ADCC or C'ADCC expression. The within and between assay variation was 7% and 16% respectively. Lymphocytotoxicity assays were performed by standard methods using guinea pig complement [16].

Statistical Analysis

All data are presented as mean $\pm$ SEM. Specific cytotoxicity expressions were compared using the unpaired Student's *t*-test.

Results

⁵¹Cr Dispersed Islet Target Cell Labelling

⁵¹Cr islet cell uptake was rapid, and sufficient radioactivity was incorporated by 30 min to permit islet cell cytotoxicity evaluations (Fig. 1). Islet cell spontaneous ⁵¹Cr release was of low magnitude permitting 2, 4 or 8 h assay incubations (Fig. 2).

Direct Rabbit Anti-Rat Islet Cell Antiserum-Mediated Cytotoxicity

To determine whether immune rabbit serum was directly toxic to islet target cells in the absence of complement, heat-inactivated rabbit anti-rat islet cell serum was coated to islet cells at dilutions of 1:1, 1:10, 1:100, 1:1000. At 1:1 and 1:10, specific cytotoxicity was 20% and 10%, respectively, but this effect was absent at dilutions greater than 1:20. Normal rabbit serum did not mediate islet cell lysis. Immunoglobulin G prepared from rabbit anti-rat islet cell serum or normal rabbit serum at islet coating concentrations of 10, 50, 100, 500 and 1000 $\mu\text{g/ml}$ did not affect antibody-mediated cytotoxicity in the absence of complement (data not shown).

Complement-Dependent Antibody-Mediated Cytotoxicity

To determine whether rabbit anti-rat islet cell serum mediated C'AMC, islets were coated with various dilutions of immune or normal rabbit serum and incubated for 1–8 h (Figs. 3 and 4). C'AMC cytotoxicity was maximal at 1:10 and 1:100 dilutions and mean half-maximal C'AMC occurred at a dilution of 1:800. Rabbit anti-rat islet cell serum was significantly more cytotoxic to islet cells than macrophage target cells, and neither normal rabbit serum, nor normal rabbit serum containing active complement, mediated islet C'AMC (Figs. 3 and 4). That C'AMC is dependent on the complement concentration is shown in Fig. 5. To determine whether rabbit anti-rat islet cell serum mediated killing via islet major histocompatibility complex antigens, two indirect experiments were performed. Immune sera-mediated C'AMC was observed to be less with macrophage target

Fig. 5. Complement dependence for complement-dependent antibody-mediated cytotoxicity (C'AMC) and complement-mediated antibody-dependent cellular cytotoxicity (C'ADCC). Various concentrations of fresh rabbit serum (complement source) were added to C'AMC assays ($n=4$) at a 1:10 dilution of rabbit anti-rat islet cell serum (●—●). Similar experiments were performed for C'ADCC ($n=4$) at an immune serum dilution of 1:1000 and a 1:40 target-effector ratio (■—■). Control experiments ($n=3$) were performed using fresh serum (complement) in the absence of immune serum (○—○)

Fig. 6. Purified IgG-mediated cytotoxicity mechanisms. Islet cells were coated with purified IgG and washed before incubation with effector systems. Rabbit anti-rat islet cell antiserum-mediated complement-dependent antibody-mediated cytotoxicity (C'AMC, ●—●, $n=7$), antibody-dependent cellular cytotoxicity (ADCC, ▲—▲, $n=5$) and complement-augmented antibody-dependent cellular cytotoxicity (C'ADCC, ■—■, $n=4$) ○—○, △—△, □—□ represent normal rabbit serum-mediated C'AMC ($n=4$), ADCC ($n=3$), and C'ADCC ($n=2$), respectively

cells than islets and both Wistar-Furth and Lewis islet target cells were lysed to a similar extent (Fig. 4). Secondly, an aliquot of rabbit anti-rat islet cell serum was doubly adsorbed using Wistar-Furth spleen cells and <10% inhibition of C'AMC against islet target cells was observed, whereas >80% lymphocytotoxicity mediated by unadsorbed serum was completely inhibited (Table 1). To determine if this C'AMC was related to

Table 1. Lymphocytotoxicity of rabbit anti-rat islet cell serum before and after Wistar-Furth spleen cell adsorption

Antiserum treatment	Undiluted	Dilution				
		1/2	1/4	1/8	1/16	1/32
Unadsorbed	8 ^a	8	8	6	2	1
One adsorption	4	1	0	0	0	0
Two adsorptions ^b	1	1	0	0	0	0

^a Lymphocytotoxicity scale: 8 (80–100%) 6 (50–79%) 4 (30–49%) 2 (20–29%) 1 (1–19%) 0 (0%)

^b After two adsorptions this antiserum remained fully cytotoxic against rat islet target cells

IgG antibody, rabbit anti-rat islet cell serum- and normal rabbit serum-derived IgG were prepared. Figure 6 shows that rabbit anti-rat islet cell serum-derived IgG mediated maximal and half maximal C'AMC at 500 and 50 μg/ml respectively, whereas normal rabbit serum-derived IgG did not mediate C'AMC.

Antibody-Dependent Cellular Cytotoxicity

To determine whether immune islet serum mediated ADCC, rabbit anti-rat islet cell serum coated target cells at dilutions of 1:10 to 1:40,000 were incubated with human non-T mononuclear cells for 4 or 8 h at target cell: effector cell ratios of 1:10 to 1:100. As shown in Fig. 7, rabbit anti-rat islet cell serum mediates ADCC at dilutions of 1:10–1:5000. The optimal target cell:effector cell ratio was 1:100, however, significant ADCC was observed always at 1:40 whereas at 1:10 or 1:20, inconsistent ADCC was noted. Incubations for 4 or 8 h showed similar ADCC responses at 1:40 target cell: effector cell ratios. Total cytotoxicity was less at 4 h than at 8 h, but after correction for direct cell-mediated cytotoxicity, ADCC was similar at both time periods. Maximal ADCC occurred at an antibody dilution of 1:10, and half-maximal ADCC at 1:300 dilution. To determine ADCC specificity to islet cells, rat macrophage target cells were used and the cytotoxicity was $10.5 \pm 2.7\%$ at a 1:10 immune serum dilution and 1:40 target cell: effector cell ratio whereas for islet target cells cytotoxicity was $51 \pm 5.1\%$. When rabbit anti-rat islet cell serum-derived IgG was coated to islet target cells, maximal and half-maximal ADCC induced by IgG occurred at approximately 200 and 50 μg/ml IgG concentrations, respectively, whereas normal rabbit serum-derived IgG did not mediate ADCC (Fig. 6).

Complement-Augmented Antibody-Dependent Cellular Cytotoxicity

To determine whether rabbit immune serum mediated C'ADCC, rabbit anti-rat islet cell serum-coated target cells were incubated with human non-T mononuclear cells and complement at immune serum dilutions of 1:10–1:40,000 for 4 h and at target cell:effector cell ratios of 1:10–1:100 (Fig. 8). Four or 8 h incubation periods resulted in similar C'ADCC results. A target:

Fig. 7. Rabbit antibody model for antibody-dependent cellular cytotoxicity (ADCC). Human non-T mononuclear cells were used as effector cells at target:effector cell ratios indicated by arrows. Incubations were for 4 h with rabbit anti-rat islet cell serum. Each data point represents at least $n = 3$

Fig. 8. Rabbit antibody model for complement-mediated antibody-dependent cellular cytotoxicity (C'ADCC). Human non-T mononuclear cells were used as effector cells at target:effector ratios indicated by arrows. Incubations were for 4 h with rabbit anti-rat islet cell serum. Each data point represents at least $n = 4$

effector ratio of 1:100 was optimal for C'ADCC. The effect of increasing complement concentrations on C'ADCC is shown in Fig. 5. Specificity of C'ADCC to islet cells was shown using macrophage target cells which were not killed at 1:1000 dilution and 1:40 target cell:effector cell ratio, whereas using islet target cells cytotoxicity was $25.6 \pm 7.1\%$. The strategy for the C'ADCC assay included variables which were corrected by subtracting both the cellular and C'AMC effects from the total cytotoxicity (see Methods). At high cytotoxic levels of C'AMC or ADCC, C'ADCC is difficult to document since C'AMC is nearly 80%–90% and direct cell-mediated cytotoxicity adds additional lysis. Thus, at dilutions of rabbit anti-rat islet cell serum 1:10 or 1:100, C'ADCC is of low magnitude whereas at dilutions which are associated with minimal C'AMC and ADCC, C'ADCC is observed. It is clear that complement can augment ADCC at a dilution of immune serum insufficient to mediate C'AMC or ADCC (Fig. 9). Expression of maximal and half maximal rabbit anti-rat islet cell serum-mediated C'ADCC was 1:1000 and

1:5000 at a target effector ratio of 1:40. As shown in Fig. 6, rabbit anti-rat islet cell serum-derived IgG mediated C'ADCC with expression of maximal and half-maximal cytotoxicity at < 100 and $1 \mu\text{g/ml}$ respectively, whereas normal rabbit serum-derived IgG did not mediate C'ADCC.

Discussion

Since the pathogenesis of Type 1 diabetes may be mediated by cytotoxic immune phenomena, *in vitro* assays capable of quantitating these mechanisms would be useful. Thus, we developed an islet target cell cytotoxicity model which characterizes assay specificity, sensitivity, incubation conditions and most importantly demonstrates multiple antibody-mediated cytotoxic mechanisms. Using this model, rabbit anti-rat islet cell serum was shown to mediate not only C'AMC but also, in collaboration with human non-T mononuclear cells, to mediate ADCC and C'ADCC. Since each of these mechanisms could be involved in the pathogenesis of insulin-

Fig. 9. Rabbit anti-rat islet cell serum-mediated cytotoxicity mechanisms: complement-dependent antibody-mediated toxicity (C'AMC, ●—●), antibody-dependent cellular cytotoxicity (ADCC, ▲—▲) and complement-mediated antibody-dependent cellular cytotoxicity (C'ADCC, ■—■). Each data point represents at least $n = 3$. These experiments illustrate the presence of C'ADCC at 1:5000 dilution in the absence of C'AMC and ADCC

dependent diabetes, these assays were further evaluated.

Normal non-cultured dispersed rat islet cells permit a maximal normal islet β cell population (70%–80%), which can be produced on a regular basis from several rodent species and man, and thus can be used in conjunction with ^{51}Cr labelling to study conventional antibody-mediated cytotoxic mechanisms. We have not directly shown that islet β cells are killed in our cytotoxicity assay system, but since triton X releases approximately 97%–100% of islet-incorporated ^{51}Cr , 85–90% islet-specific C'AMC suggests that β cells are the major target cell type evaluated in these assays assuming all islet cells have similar ^{51}Cr uptake. Recent results from other laboratories also suggest islet β cell killing in cultured islet target cell systems [3, 6].

Other variables also exist for complement-dependent antibody-mediated cytotoxicity assays, such as cross-reactivity between human antibodies and rodent islet β cells. Both antibody islet cell binding and cytotoxic cross-reactivities have been well-documented [4–7, 17]. Although the pathogenic relationships of islet cell antibody binding and cytotoxicity remain unclear, one recent report describes monoclonal antibodies, some of which bind only rat islet tumour cells, whereas others are also cytotoxic [18].

Specific C'AMC was shown using the rabbit anti-rat islet cell serum model antibody system. Similar observations have been reported using human sera derived from insulin-dependent diabetic patients [4–7], however, DEAE-purified IgG has not been investigated. Our studies directly show that C'AMC is mediated by

the IgG fraction of the rabbit antisera. Since we show also that this antiserum has non-IgG-mediated cytotoxic effects at high concentrations, human sera also should be examined for IgG-mediated cytotoxicity.

Our studies suggest that rabbit anti-rat islet cell serum-induced C'AMC is not related to islet major histocompatibility antigens since rabbit immune sera was equally effective against islet cells of different RTI classes (Wistar-Furth and Lewis) but was less effective against rat macrophage target cells. Since other cell types have not yet been examined these conclusions should be considered preliminary. After adsorption using Wistar-Furth spleen cells, the rabbit antisera remained cytotoxic to islet cells despite the abolition of lymphocytotoxicity. The lymphocyte and macrophage cytotoxicity initially present could be related to passenger immune cells present in islet cell preparations and/or MHC antigens on islet cells [19].

Islet ADCC mechanisms have not been reported previously although insulin-dependent patients are described to have increased numbers of K cells and increased K cell cytotoxicity against non-islet target cells [20, 21]. Others have predicted ADCC to be an important mechanism in the pathogenesis of diabetes [1]. We have reported preliminary data of ADCC in insulin-dependent diabetic patients [4]. Some normal subjects' mononuclear cells did not mediate ADCC at target cell:effector cell ratios of 1:10 and 1:20; but in these earlier studies we used target:effector cell ratios that were too low as indicated by the present data. The finding of rabbit anti-rat islet cell serum-mediated ADCC in the current study serves as a positive control for the gen-

eral absence of ADCC in sera derived from insulin-dependent diabetic patients reported in our subsequent studies [8, 22].

Complement-augmented ADCC has been described in model systems and it has been suggested that complement enhances binding of cellular effectors to antibody-bound target cells [23–25]. The relevance of C'ADCC mechanisms to disease pathogenesis is unclear. The relevance of C'ADCC to insulin-dependent diabetes is suggested by our recent observations in humans [8, 22], and our documentation that human effector cells can effectively collaborate with antibody and complement to kill islet cells. For ADCC and C'ADCC, DEAE-purified IgG was shown to mediate islet cell cytotoxicity, thus eliminating non-specific serum toxic factors as a potential mechanism.

The nature of the cytotoxic reactivities expressed by the three antibody mechanisms studied suggests that (a) each antibody mechanism has a unique minimum serum dilution for maximal cytotoxicity expression, i.e., 1:100 for C'AMC, 1:10 for ADCC, and 1:1000 for C'ADCC, (b) each antibody mechanism has a unique half-maximal immune serum dilution for cytotoxicity expression, i.e., 1:800 for C'AMC, 1:300 for ADCC and 1:5000 for C'ADCC and (c) although C'AMC shows the greatest capacity for islet cell lysis, C'ADCC appears to be the most sensitive cytotoxic mechanism. Since cellular infiltrates are observed in recent onset insulin-dependent diabetic patients' islets, cellular mechanisms in collaboration with antibody (ADCC or C'ADCC), or cellular mechanisms alone, may be important pathogenic factors. The relative roles of C'AMC, ADCC and C'ADCC with respect to insulin-dependent diabetes can be evaluated in detail using the *in vitro* assay systems described.

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