# Interleukin 2 Activates BB/W Diabetic Rat Lymphoid Cells Cytotoxic to Islet Cells

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**SUMMARY**

**We compared the cytotoxic effects to islet cells of lymphoid cells from diabetic and diabetes-resistant (DR) BioBreeding/Worcester (BB/W) rats with a $^{51}$Cr-release assay to detect lysis of normal rat islet cells. Splenic lymphoid cells from diabetic rats were more cytotoxic to islet cells (11.3 ± 3.8%) than were lymphoid cells from DR rats (4.0 ± 2.6%). This difference was amplified by incubating the lymphoid cells for 20 h with 5 μg/ml concanavalin A (ConA); islet cell lysis was 39.3 ± 4.5% by ConA-activated diabetic cells and 9.6 ± 2.7% by ConA-activated DR cells. The cytotoxic lymphoid cells were identified as natural killer (NK) cells, because treatment of diabetic lymphoid cells with anti-asialo GM1 serum and complement selectively removed a monoclonal antibody–defined subset of NK cells (OX8+), and the NK-depleted lymphoid cells were not cytotoxic to either islet or NK-sensitive YAC-1 cells, even after culture with ConA. Of several lymphokine products of ConA-stimulated lymphoid cells, interleukin 2 (IL-2), but not interleukin 1 or interferon-γ, significantly activated splenic lymphoid cells cytotoxic to islet cells, and the lymphoid cells from diabetic rats were more sensitive to IL-2 (3 U/ml) than were the cells from DR rats (30 U/ml). This study reveals the presence of ConA- and IL-2–responsive islet cytotoxic NK cells in the diabetic BB/W rat and suggests that IL-2 activation of NK cells may contribute to islet β-cell destruction and diabetes in this animal. *Diabetes* 36:1217–22, 1987**

Several lines of evidence suggest a cell-mediated immune pathogenesis for the development of insulin-dependent diabetes mellitus (IDDM) in the BioBreeding (BB) rat (1). Histological studies of acutely diabetic BB rats show intense infiltration of pancreatic islets (insulitis) by mononuclear cells, mostly activated lymphocytes and macrophages, followed by destruction of insulin-secreting β-cells (2–4). Furthermore, adoptive transfer of both insulitis and diabetes has been accomplished by injection of concanavalin A (ConA)-activated splenic lymphoid cells from acutely diabetic BB/Worcester (BB/W) rats into young diabetes-prone (DP) BB/W rats (5), into a diabetes-resistant (DR) subline of BB/W rats (6), or into major histocompatibility complex (MHC)-compatible Wistar-Furth (WF) rats pretreated with cyclophosphamide (7).

Because the lectin ConA is a potent stimulator of lymphokine production by T-lymphocytes (8), the adoptive transfer experiments suggest that lymphokine-dependent cells might be involved in islet β-cell damage. To test this hypothesis, we cultured splenic lymphoid cells from BB/W diabetic rats with ConA and the lymphokines interleukin 2 (IL-2), interferon-γ (IFN-γ), and interleukin 1 (IL-1) and then tested the cells for cytotoxic activity against pancreatic islet cells in vitro with a previously described $^{51}$Cr-release assay to detect cell-mediated islet cell lysis (9). Our results indicate that ConA and IL-2 are potent activators of islet-directed cytotoxic lymphoid cells from diabetic rats and that these cells have the properties of natural killer (NK) cells.

## MATERIALS AND METHODS

**Animals.** Female BB/W rats were obtained from the National Institutes of Health contract colony maintained at the University of Massachusetts Medical School, Worcester, Massachusetts. Diabetic rats were treated with a single daily injection of 1–2 U protamine zinc insulin (Lilly, Indianapolis, IN) and were studied within 12 days of diagnosis (age 80–120 days). Diabetes was defined as the presence of glucosuria (2–4+ with Tes-Tape) and plasma glucose >200 mg/dl. DR rats, age matched to the diabetic rats, were obtained from BB/W sublines bred for resistance to diabetes.

**Splenic lymphoid cell preparation and culture.** Lymphoid (mononuclear) cells were isolated from BB/W rat spleens by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gra-

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dient centrifugation as described previously (10). The splenic lymphoid cells from the diabetic and DR rats were used as effector cells in cytotoxicity assays, either freshly isolated or after incubation for 20 h at 37°C in 95% air/5% $CO_2$ at 2 × $10^6$ cells/ml in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 6 mM HEPES, 5 × $10^{-5}$ M β-mercaptoethanol, and 10% heat-inactivated fetal calf serum (complete RPMI medium), in the presence or absence of 5 μg/ml ConA (Sigma, St. Louis, MO) or various lymphokines.

**Lymphokine preparations.** Purified human IL-1 and IL-2 were purchased from Collaborative Research (Lexington, MA). For the IL-1 preparation, 2–3 U stimulated maximum [$^3$H]thymidine incorporation by normal mouse thymocytes. For the IL-2 preparation, 10 U produced a 21-fold increase in cell number over 3 days in a cell growth assay with a mouse T-lymphocyte cell line (CTLL). Rat recombinant IFN-γ was kindly provided by Dr. P. H. van der Meide, Institute of the Division for Health Research, Rijswikj, The Netherlands.

**NK cell depletion.** To assess the effects of NK cell depletion on the cytotoxic activity of BB/W rat lymphoid cells, the splenic lymphoid cells from diabetic and DR rats were incubated (10 × $10^6$ cells) for 1 h at 4°C in 1 ml serum-free RPMI medium containing a 1:50 dilution of anti-asialo GM1 rabbit serum, γ-globulin fraction (~10 mg/ml) (Wako Chemicals USA, Dallas, TX). This antiserum contains antibody to a neutral glycosphingolipid (asialo GM1) present in high quantity on the surface of NK cells (11). Control incubations were carried out in normal rabbit serum (1:50). The cells were then washed twice in serum-free RPMI and incubated for 45 min at 37°C in 500 μl of a 1:10 dilution of Low Tox H rabbit complement (Cedarlane, Hornby, Ontario, Canada) followed by an additional 45 min at 37°C with fresh complement. The cells were washed three times with complete RPMI medium, viable cells were counted, aliquots (~2 × $10^6$ cells) were phenotyped by monoclonal antibody–immunofluorescence assay, and the remaining cells were incubated (2 × $10^6$/ml) for 20 h in complete RPMI medium supplemented with 5 μg/ml ConA before testing in cytotoxicity assays.

**Phenotyping of lymphoid cells.** Splenic lymphoid cells from BB/W rats were phenotyped by immunofluorescence staining, with mouse anti-rat monoclonal antibodies W3/13, OX19, W3/25, and OX8 (Accurate Chemical and Scientific, Westbury, NY). W3/13 reacts with all T-lymphocytes, NK cells, polymorphonuclear cells, hemopoietic stem cells, and plasma cells but not with B-lymphocytes. OX19 reacts with T-lymphocytes only. W3/25 reacts with helper T-lymphocytes ($T_h$) and macrophages. OX8 reacts with cytotoxic/suppressor T-lymphocytes ($T_{c/s}$) and NK cells. Aliquots of 2 × $10^6$ splenic lymphoid cells from diabetic and DR rats were incubated for 30 min at 4°C separately and with monoclonal antibodies W3/13, OX19, W3/25, and OX8 (all at 1:20 dilution) in 100 μl phosphate-buffered saline (pH 7.4) containing 10 mg/ml bovine serum albumin and 0.2 mg/ml sodium azide (assay buffer). The cells were then washed twice and incubated for another 30 min at 4°C in 100 μl of a 1:20 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, with no cross-reaction to rat IgG (Cooper, West Chester, PA). Immunoglobulin-positive B-lymphocytes were labeled directly by incubation for 30 min at 4°C in 100 μl of a 1:20 dilution of FITC-conjugated mouse anti-rat immunoglobulin, F(ab')$_2$ fragment (Accurate Chemical and Scientific). After incubation with the FITC-conjugated antibodies, the cells were washed twice, fixed in 4% paraformaldehyde, and analyzed on an EPICS V flow cytometer (Coulter Electronics, Hialeah, FL) according to relative fluorescence intensity. Background fluorescence, measured by incubating cells with FITC-conjugated goat anti-mouse IgG only, averaged 4%.

**Target cells.** Islet donors were inbred WF rats, MHC compatible (RT1$^u$) with the BB/W rats that provided the effector lymphoid cells. Islets were isolated by collagenase enzyme digestion of the pancreas (12), with ductal injection of the enzyme (13). The islets were dissociated into single cells by incubation in $Ca^{2+}$/$Mg^{2+}$-free Hanks' balanced salt solution supplemented with 0.5 mg/ml trypsin and 0.2 mg/ml EDTA (trypsin-EDTA) solution at 37°C for 10 min. Cell viability was >85%, and 80–90% of the cells were islet endocrine cells, of which ~80% were insulin-containing β-cells (14). WF splenic lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation. The RIN (clone 5F) cell line derived from an NEDH rat (RT1$^9$) islet cell tumor synthesizes and secretes primarily insulin (15). RIN cells in monolayer culture were dissociated by incubation in trypsin-EDTA solution at 37°C for 5 min and washed twice in complete RPMI medium before use as target cells. The $GH_3$ rat pituitary cell line (16), which also grows in monolayer culture, was treated similarly. A cell line (YAC-1) derived from a virus-induced mouse lymphoma and very sensitive to lysis by NK cells (17) was maintained in suspension culture in complete RPMI medium and also used as a target cell.

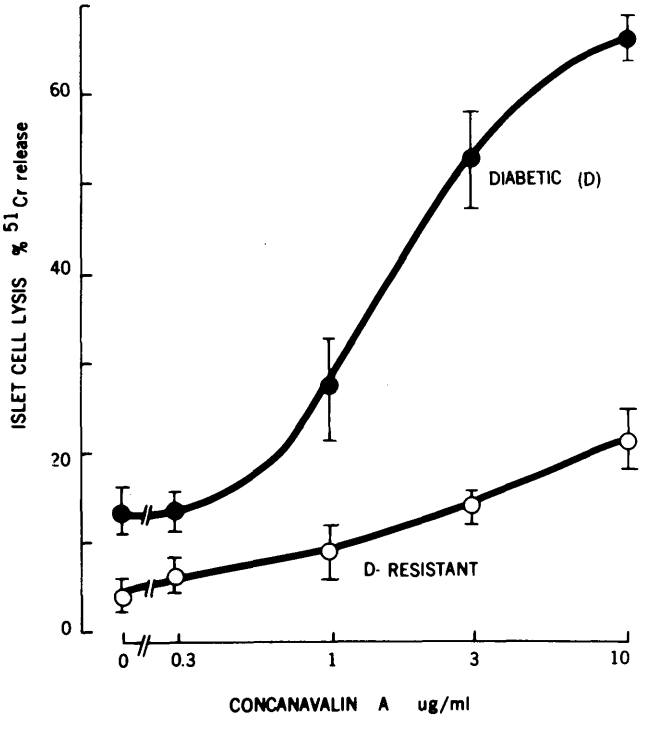


**FIG. 1. Splenic lymphoid cells from diabetic and diabetes-resistant rats, either freshly isolated [0 concanavalin A (ConA)] or incubated for 20 h with 0.3–10 μg/ml ConA, were used as effector cells (5 × $10^5$/well) in 6-h cytotoxicity assay with $^{51}$Cr-labeled Wistar-Furth rat islet cells ($10^4$/well) as targets. Means ± SE for percent $^{51}$Cr release from targets (islet cell lysis) are shown for 5 diabetic and 3 diabetes-resistant rats.**

TABLE 1
Target cell specificities of BB/W rat splenic lymphoid cells

| Target cells | n | Cytotoxicity (% $^{51}$Cr release) | | | |
|---|---|---|---|---|---|
| | | Freshly isolated cells | | ConA-activated cells | |
| | | D | DR | D | DR |
| Islet (WF rat) | 8 | 11.3 ± 3.8* | 5.0 ± 2.6 | 39.3 ± 4.5† | 10.6 ± 2.7 |
| Spleen (WF rat) | 3 | 2.9 ± 2.2 | 3.7 ± 3.0 | 0.9 ± 3.1 | 2.5 ± 0.5 |
| Islet (RIN5F) | 6 | 13.1 ± 4.1‡ | 3.6 ± 2.7 | 34.2 ± 4.4† | 8.4 ± 2.2 |
| Pituitary ($GH_3$) | 3 | ND | ND | 2.9 ± 0.8 | 0.8 ± 0.4 |
| YAC-1 | 6 | 30.7 ± 5.2‡ | 10.1 ± 4.2 | 50.4 ± 5.5† | 18.9 ± 3.3 |

Splenic lymphoid cells from diabetic (D) and diabetes-resistant (DR) rats were freshly isolated or incubated for 20 h with 5 μg/ml concanavalin A (ConA), washed, then incubated for 6 h as effectors (5 × $10^5$/well) with different $^{51}$Cr-labeled cells ($10^4$/well) as targets. Values are means ± SE for percent $^{51}$Cr release from targets. WF, Wistar-Furth. ND, not determined. *$P < .05$, †$P < .001$, and ‡$P < .02$ vs. similarly prepared DR effector cells (paired $t$ test).

**$^{51}$Cr-release assay.** Lysis of target cells was detected by an assay for the release of $^{51}$Cr from damaged cells. Targets (2 to 3 × $10^6$ cells) were labeled with 200 μCi [$^{51}$Cr]sodium chromate (New England Nuclear, Boston, MA) in 0.3 ml complete RPMI medium at 37°C for 90 min, then washed four times, resuspended in complete RPMI medium (0.2 × $10^6$ cells/ml), and seeded at $10^4$ cells/well in 96-well ½-area microculture plates (Costar, Cambridge, MA). Effector splenic lymphoid cells were seeded in the wells (0.5 to 5.0 × $10^5$ cells in 100 μl) in quadruplicate. Medium only was added to a set of target cells to determine spontaneous cell lysis, and 4% Triton X-100 was added to determine total (100%) lysis. The plates were centrifuged (100 × $g$ for 3 min) and incubated at 37°C for 6 h. An aliquot (100 μl) of supernatant was collected from each well and counted in an LKB 1272 γ-counter (LKB, Gaithersburg, MD). Spontaneous release ranged from 5 to 15%. Percent specific $^{51}$Cr release: 100 × [(sample counts/min) − (spontaneous counts/min)] ÷ [(total counts/min) − (spontaneous counts/min)].

**Statistical analyses.** Results are expressed as means ± SE. Data were analyzed by Student's $t$ test for unpaired samples or for paired samples if so stated.

## RESULTS

**Effects of ConA.** Figure 1 shows the dose-dependent stimulatory effects of ConA to activate BB/W rat splenic lymphoid cells cytotoxic to rat islet cells. Freshly isolated splenic lymphoid cells from diabetic rats were significantly more cytotoxic to islet cells than were splenic cells from DR rats (13.8 ± 2.4 vs. 4.3 ± 1.7%, $P < .01$). Incubation of the lymphoid cells for 20 h with ConA amplified these differences greatly. The threshold concentration of ConA that significantly increased islet cell–directed cytotoxicity was 1 μg/ml for diabetic cells and 3 μg/ml for DR cells; after incubation with 10 μg/ml ConA, diabetic cells were three times more cytotoxic to islet cells (66.6 ± 3.5%) than were DR cells (21.6 ± 3.4%, $P < .001$).

The target cell specificities of the freshly isolated and 20-h ConA-activated splenic lymphoid cells from diabetic and DR rats are shown in Table 1. Similar to the experiments shown in Fig. 1, WF rat islet cells were significantly more sensitive to lysis by freshly isolated diabetic than DR lymphoid cells (11.3 ± 3.8 vs. 5.0 ± 2.6%, $P < .05$), and this difference was increased by using lymphoid cells activated

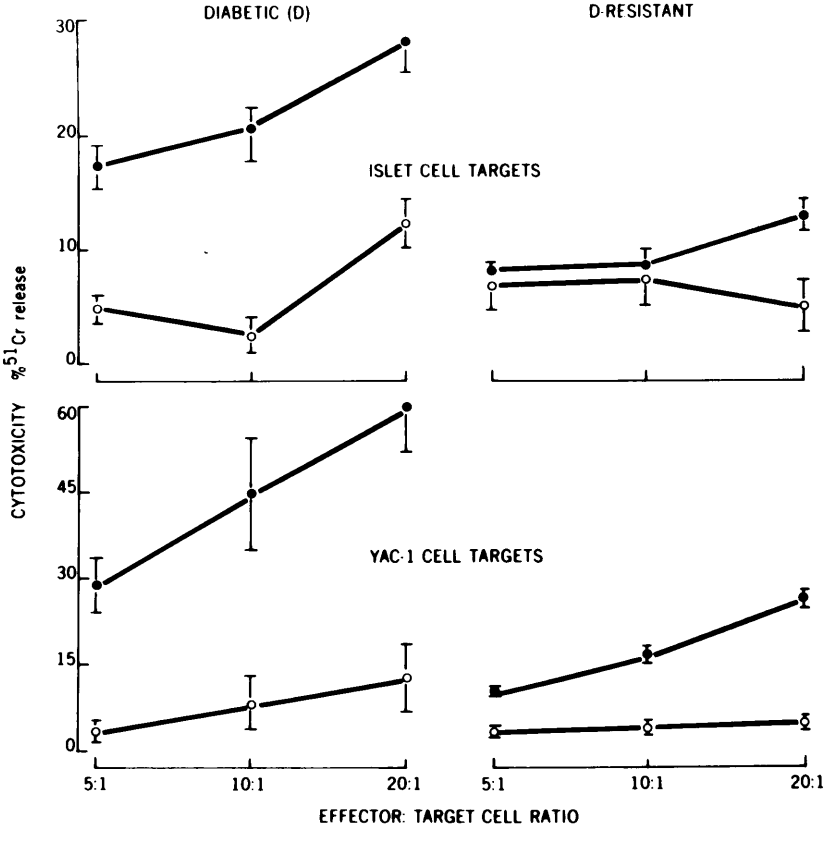


**FIG. 2. Splenic lymphoid cells from diabetic and diabetes-resistant rats were incubated with anti-natural killer cell rabbit serum anti-asialo GM1 (αASGM1, ○) and complement or normal rabbit serum and complement (control, ●). Cells were washed, incubated for 20 h with 5 μg/ml concanavalin A, and then used as effector cells in 6-h cytotoxicity assays with either $^{51}$Cr-labeled Wistar-Furth rat islet cells or YAC-1 cells as targets ($10^4$/well). Means ± SE for percent $^{51}$Cr release from targets (cytotoxicity) are shown for 5 diabetic and 4 diabetes-resistant rats.**

TABLE 2
Effect of anti-NK cell serum on BB/W rat lymphoid cell subsets

| Lymphoid cells | *n* | Percentage of cells staining with | | | | |
|---|---|---|---|---|---|---|
| | | W3/13 (T + NK) | OX19 (T) | OX8 ($T_{c/s}$ + NK) | W3/25 ($T_h$) | B-lymphocyte |
| Diabetic | | | | | | |
| Control | 4 | 52 ± 1 | 28 ± 2 | 31 ± 1 | 29 ± 1 | 31 ± 2 |
| α-ASGM1 | 4 | 37 ± 2* | 26 ± 2 | 13 ± 5* | 29 ± 5 | 41 ± 1 |
| Diabetes resistant | | | | | | |
| Control | 4 | 69 ± 4 | 74 ± 6 | 24 ± 5 | 43 ± 5 | 9 ± 4 |
| α-ASGM1 | 4 | 67 ± 5 | 64 ± 4 | 19 ± 3 | 51 ± 6 | 18 ± 4 |

Splenic lymphoid cells from diabetic and diabetes-resistant rats were incubated with anti-natural killer (NK) cell rabbit serum, anti-asialo GM1 (α-ASGM1) and complement, or normal rabbit serum and complement (control). Cells were then washed and incubated with mouse monoclonal antibodies to different rat lymphoid cell subsets shown, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antiserum. B-lymphocytes were stained directly with FITC-conjugated mouse anti-rat IgG, F $(ab')_2$. Values are means ± SE for percentage of fluorescent cells. T, T-lymphocyte; $T_{c/s}$, cytotoxic/suppressor T-lymphocyte; $T_h$, helper T-lymphocyte. *$P < .02$ vs. diabetic control cells.

by ConA (39.3 ± 4.5% by ConA-activated diabetic cells vs. 10.6 ± 2.7% by ConA-activated DR cells, $P < .001$). Also, studies with DR rat islet cells as targets gave results nearly identical to those seen with WF rat islet cell targets. In contrast to islet cell targets, WF spleen cells were not significantly lysed by either fresh or ConA-activated diabetic or DR lymphoid cells. In addition to normal MHC-compatible WF ($RT1^u$) rat islet cells, the MHC-incompatible NEDH rat ($RT1^9$) islet cell tumor line (RIN5F) was sensitive to lysis by diabetic lymphoid cells (13.1 ± 4.1% by diabetic effectors vs. 3.6 ± 2.7% by DR effectors, $P < .02$); this difference was amplified with ConA-activated lymphoid effectors (34.2 ± 4.4% by diabetic cells and 8.4 ± 2.2% by DR cells, $P < .001$). In contrast, a rat pituitary cell line ($GH_3$) was not sensitive to lysis by ConA-activated diabetic cells. Finally, freshly isolated diabetic lymphoid cells were strongly cytotoxic to a cell line (YAC-1) sensitive to lysis by NK cells (30.7 ± 5.2% by diabetic cells and 10.1 ± 4.2% by DR cells, $P < .02$); this difference was increased with ConA-activated lymphoid effectors (50.4 ± 5.5% by diabetic cells and 18.9 ± 5.3% by DR cells, $P < .02$).

**Effects of an anti-asialo GM1 serum.** To demonstrate the NK cell identity of the cytotoxic lymphoid cells, incubations were performed with anti-asialo GM1 serum, which preferentially reacts with NK cells (11), and complement. After sufficient time for specific complement-mediated cytolysis, the remaining cells were incubated for 20 h with ConA to activate cytotoxic cells. Figure 2 shows that anti-asialo GM1 serum reduced or eliminated the cytotoxic effects of diabetic and DR lymphoid cells to both islet and YAC-1 cells. For effector cell–to–target cell ratios of 10:1 and 5:1, prior treatment of the diabetic effector cells with anti-asialo GM1 serum and complement reduced lymphoid cell cytotoxicity to both islet and YAC-1 cell targets to insignificant (≤4–8%) levels. The anti-asialo GM1 serum used to remove NK cells from the splenic lymphoid cell preparations is not entirely specific for NK cells; there is also some binding to monocytes and immature thymocytes in the rat (18). Therefore, we monitored the effects of treatment with anti-asialo GM1 serum and complement on the different lymphoid cell subsets present in the mononuclear cells isolated from spleens of diabetic and DR rats. Table 2 shows that treatment of diabetic splenic cells with anti-asialo GM1 serum significantly decreased the monoclonal antibody–defined W3/13+ (T + NK) cells as well as the OX8+ ($T_{c/s}$ + NK) cells, whereas OX19+ (total T) cells and W3/25+ ($T_h$) cells were not significantly changed, and the percentage of immunoglobulin-positive B-lymphocytes was increased. These findings, taken together with the report that virtually all OX8+ cells in BB/W DP and diabetic rats are NK cells (19), indicate that anti-asialo GM1 serum selectively removed NK cells from the splenic lymphoid cells of diabetic rats. Treatment of splenic lymphoid cells from DR rats with anti-asialo GM1 serum also reduced the OX8+ cell subset; however, the decrease was not significant (Table 2). This is in agreement with the demonstration that the contribution of NK cells to the OX8+ subset is much smaller in DR than in DP or diabetic rats, and that the large majority of OX8+ ($T_{c/s}$ + NK) cells are $T_{c/s}$ cells in the DR rat and NK cells in DP and diabetic rats (19).

**Effects of lymphokines.** To examine possible mechanisms whereby ConA might activate cytotoxic NK cells in a mononuclear cell preparation isolated from the spleens of diabetic rats, we tested several lymphokine products of ConA-activated lymphoid cells. Only IL-2 could reproduce the effects

TABLE 3
Effects of lymphokines on cytotoxicity of BB/W rat lymphoid cells to islet cells

| Agent | Concn | *n* | Cytotoxicity (% $^{51}Cr$ release) | |
|---|---|---|---|---|
| | | | Diabetic cells | Diabetes-resistant cells |
| None | | 6 | 8.3 ± 1.6 | 6.8 ± 1.0 |
| Concanavalin A | 5 μg/ml | 6 | 35.9 ± 4.4* | 15.1 ± 3.0† |
| Interleukin 2 | 10 U/ml | 6 | 26.3 ± 4.5‡ | 10.5 ± 1.4 |
| Interferon-γ | 10 U/ml | 3 | 6.5 ± 0.4 | 5.8 ± 1.6 |
| | 100 U/ml | 3 | 5.2 ± 0.8 | 5.0 ± 0.9 |
| | 1000 U/ml | 3 | 5.0 ± 0.5 | 5.2 ± 1.2 |
| Interleukin-1 | 1 U/ml | 3 | 7.8 ± 1.7 | 7.5 ± 2.2 |
| | 10 U/ml | 3 | 9.1 ± 1.6 | 8.1 ± 2.6 |
| | 30 U/ml | 3 | 8.3 ± 0.8 | 7.7 ± 3.2 |

Splenic lymphoid cells from diabetic and diabetes-resistant rats were incubated for 20 h in the presence or absence of agents shown, washed, then incubated for 6 h as effectors ($5 \times 10^5$/well) with $^{51}Cr$-labeled Wistar-Furth rat islet cells ($10^4$/well) as targets. Values are means ± SE for percent $^{51}Cr$ release from targets. *$P < .01$, †$P < .05$, and ‡$P < .02$ vs. no agent.

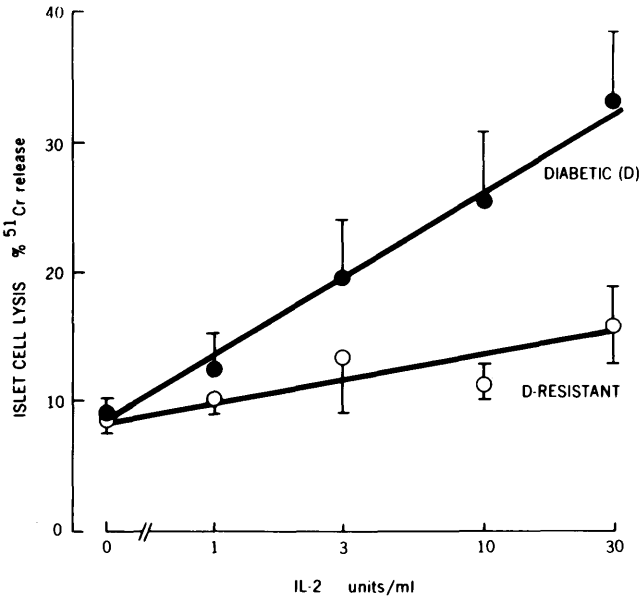


**FIG. 3. Splenic lymphoid cells from diabetic and diabetes-resistant rats were incubated for 20 h with and without 1–30 U/ml interleukin 2 (IL-2), washed, then incubated as effector cells (5 × $10^5$/well) in 6-h cytotoxicity assay with $^{51}$Cr-labeled Wistar-Furth rat islet cells ($10^4$/well) as targets. Means ± SE for percent $^{51}$Cr release from targets (islet cell lysis) are shown for 6 diabetic and 4 diabetes-resistant rats.**

of ConA (Table 3). IL-2 (10 U/ml) significantly increased the cytotoxicity of diabetic lymphoid cells to islet cells, from 8.3 ± 1.6 to 26.3 ± 4.5% ($P < .02$). The effect of IL-2 on lymphoid cells from DR rats was not significant. IL-1 (1–30 U/ml) and rat IFN-γ (10–1000 U/ml) failed to activate cytotoxic cells from either diabetic or DR rats. The dose-response relationships for the effects of IL-2 are shown in Fig. 3. IL-2 induced a concentration-dependent increase in cytotoxicity of diabetic and DR lymphoid cells to islet cells. Lymphoid cells of diabetic rats were more sensitive to the stimulatory effect of IL-2 than were the cells of DR rats; 3 U/ml IL-2 stimulated a twofold increase in the cytotoxicity of diabetic cells (19.3 ± 4.9 vs. 8.8 ± 1.5%, $P < .05$), whereas 30 U/ml IL-2 were required to produce a significant increase in cytotoxicity of DR cells (15.9 ± 2.9 vs. 8.5 ± 0.9%, $P < .05$). The time course of the stimulatory effects of IL-2 is shown in Fig. 4. After 10 h of incubation with 30 U/ml IL-2, diabetic lymphoid cells were significantly more cytotoxic to islet cells than were DR cells (18.6 ± 2.5 vs. 10.1 ± 2.8%, $P < .05$). The maximum stimulatory effect on diabetic cells was seen at 48 h and persisted for 72 h of incubation with IL-2. Diabetic cells incubated for 36 h with IL-2 (10 U/ml) were also significantly more cytotoxic to YAC-1 target cells (26.3 ± 3.3%) than were diabetic cells incubated in the absence of IL-2 (11.4 ± 1.2%), at an effector-to-target cell ratio of 20:1.

## DISCUSSION

We have previously reported that splenic lymphoid cells from BB/W diabetic and DP rats are capable of lysing pancreatic islet cells in vitro (9) and that these lymphoid cells have the characteristics of NK cells (10). In this study, we report that the islet-directed cytotoxicity of splenic lymphoid cells from diabetic rats is greatly increased after incubation with either ConA, a lectin that activates lymphoid cells, or IL-2, a lymphokine produced by activated lymphocytes (8).

ConA-activated cells from diabetic rats were cytotoxic to both syngeneic BB/W DR rat islet cells and MHC-compatible WF ($RT1^u$) rat islet cells. Similar cytotoxic effects were also observed with MHC-incompatible rat islet cell line RIN5F and NK-sensitive cell line YAC-1. The cytotoxic lymphoid cells in diabetic rats were identified as NK cells because treatment with anti-asialo GM1 serum and complement selectively removed a monoclonal antibody–defined subset of NK cells (OX8+) without change in the proportion of T-lymphocytes (OX19+), and the NK-depleted lymphoid cells were not cytotoxic to either islet cells or YAC-1 cells.

Low concentrations of IL-2 (1–10 U/ml) were capable of activating islet-directed cytotoxic cells from diabetic rats, whereas even high concentrations of rat IFN-γ failed to induce similar activity. In the mouse, IL-2 alone was reported to be sufficient for induction of cytotoxic NK cells from either spleen or bone marrow, whereas IFN-γ had no significant effect on cells from either source (20). In human studies, IFN-γ had little or no ability to augment NK activity of peripheral blood lymphocytes, and Sayers et al. concluded that IL-2–induced augmentation of NK activity is due to the direct action of IL-2 on large granular lymphocytes (NK cells) (21). The rapid induction of cytotoxic activity by IL-2 observed in this study (10–48 h) is consistent with an effect of Il-2 at some stage in NK cell differentiation (20).

The greater sensitivity to IL-2 of lymphoid cells from diabetic than DR rats suggests that increased numbers of NK cells alone do not account for the observed differences in cytotoxicity. In agreement with this observation, Woda and Biron (22) demonstrated that NK cells isolated from BB/W DP rat spleens showed threefold more lysis of NK-sensitive YAC-1 cells than did an equal number of NK cells from DR

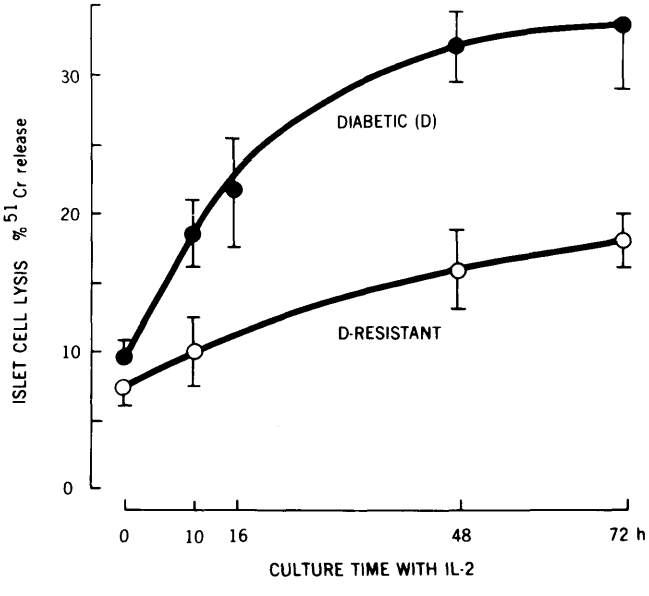


**FIG. 4. Splenic lymphoid cells from diabetic and diabetes-resistant rats were incubated for 0–72 h with 30 U/ml interleukin 2 (IL-2), washed, then incubated as effector cells (5 × $10^5$/well) in 6-h cytotoxicity assay with $^{51}$Cr-labeled Wistar-Furth rat islet cells ($10^4$/well) as targets. Means ± SE for percent $^{51}$Cr release from targets (islet cell lysis) are shown for 5–9 diabetic or diabetes-resistant rats.**

animals. These authors speculated that the enhanced activity of NK cells from DP rats might be due to increased levels of interferon or IL-2. Our findings support these proposals, because we have shown that diabetic rats possess cytotoxic lymphoid cells with the characteristics of NK cells and that these cells, on activation with IL-2, become strongly lytic to islet cells.

Recently, Like et al. (23) demonstrated that T-lymphocytes (OX19+, OX8−), as well as NK cells (OX8+, OX19−), may be necessary for the development of diabetes in BB/W rats, because removal of either cell type by administration in vivo of OX19 or OX8 monoclonal antibody prevented the appearance of diabetes in DP animals. Therefore, activated T-lymphocytes, present in the early insulitis lesion in the BB rat (3), may provide the IL-2 necessary to activate NK cells in the mononuclear infiltrate, and these activated NK cells could directly contribute to islet cytotoxicity. The lack of MHC restriction by the activated NK cells is not inconsistent with islet cell destruction in diabetic BB rats, because allogeneic islets are susceptible to tissue-specific damage after grafting to DP BB rats (24,25). Also, we have previously reported that islet β-cells can be damaged by NK cells in vitro (9); however, it remains to be determined whether this is selective for β-cells.

Although the precise triggering events that initiate autoimmune diseases, including IDDM, are unknown, there is evidence to support the hypothesis that lymphoid cells responsive to IL-2 are important as autoimmune effectors (26). Similarly, our findings suggest that IL-2–dependent lymphoid cells may mediate islet β-cell destruction in the BB/W diabetic rat. Other observations support this interpretation. First, passive transfer of diabetes can be effected with ConA-activated spleen cells from diabetic rats (5–7). Second, treatment of DP rats with recombinant human IL-2 may accelerate the onset and increase the incidence of spontaneous diabetes (27). Third, treatment of DP rats with cyclosporin A (CsA), an immunosuppressive agent whose main action is to inhibit lymphokine production (28), will prevent the appearance of diabetes in rats (29,30). Finally, we have found that CsA in vitro can inhibit ConA-stimulated production of IL-2 as well as activation of islet-directed cytotoxic lymphoid cells isolated from DP rats (31).

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