Suppression of diabetes mellitus in the non-obese diabetic (NOD) mouse by an autoreactive (anti-I-A^{g7}) islet-derived CD4⁺ T-cell line

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Summary. The non-obese diabetic (NOD) mouse is a spontaneous model of human insulin-dependent diabetes mellitus. Both CD4⁺ and CD8⁺ T cells infiltrate the pancreatic islets of NOD mice prior to beta-cell destruction. T-cell lines isolated from the islets of NOD mice are tools for studying the pathogenesis of insulin-dependent diabetes mellitus. During attempts to generate such lines we isolated an autoreactive CD4⁺ T-cell line, designated C2, from the 'insulinitis' lesion of a 20-week-old female non-diabetic NOD/WEHI mouse. Islet T cells were propagated by the addition of interleukin-2 and re-exposure every 2 weeks to whole NOD islets and irradiated NOD spleen cells as antigen presenting cells. C2 cells proliferated up to 100-fold upon exposure to NOD antigen presenting cells but did not respond to whole NOD islets or antigen presenting cells from allogeneic mouse strains. Proliferation of C2 cells to NOD antigen presenting cells was blocked by a monoclonal antibody against the unique class II MHC molecule of NOD, I-A^{g7}. In response to NOD antigen presenting cells, C2 cells secreted interferon- γ , tumour necrosis factor- α

and interleukin-6 but no detectable interleukin-2, interleukin-4 or interleukin-10, a pattern of cytokine secretion more characteristic of Th₁ CD4 cells. C2 cells displayed significant cytotoxicity in a redirected lysis assay. To explore a possible role for autoreactive T cells in the pathogenesis of autoimmune diabetes, C2 cells were injected i. v. into female NOD mice that had received cyclophosphamide to accelerate development of diabetes. Although not different at day 10, by day 16 the frequency of diabetes (blood glucose > 15 mmol/l) in mice given cyclophosphamide and C2 cells was significantly less than in mice given cyclophosphamide alone ($p < 0.05$). These results are consistent with the 'suppressor' function of autoreactive T-cells previously reported and with the concept that autoreactive T cells modulate autoimmune beta-cell destruction in the NOD mouse.

Key words: Insulin-dependent diabetes mellitus, non-obese diabetic (NOD) mouse, autoreactive T-cell clones, suppressor T cells.

The non-obese diabetic (NOD) mouse is a model for studying the pathogenesis of insulin-dependent diabetes mellitus. Both CD4⁺ and CD8⁺ T cells infiltrate the pancreatic islets of NOD mice prior to the onset of beta-cell destruction [1, 2] and islet-reactive CD4⁺ and CD8⁺ T-cell clones isolated from NOD mice adoptively transfer diabetes [3–6]. However, protection against diabetes may also be conferred by T-cell clones isolated from the NOD 'insulinitis' lesion [6, 7]. This suggests that the process of beta-cell destruction is a balance between T cells with cytotoxic and suppressor functions.

The syngeneic mixed lymphocyte reaction (SMLR), due to recognition of self MHC class II by autoreactive CD4⁺ T cells, is involved in the generation of T cells capable of suppressing the proliferative and cytotoxic responses of fresh T cells [8]. Impaired suppressor T-cell function in the NOD mouse is suggested by a reduced SMLR compared to non-autoimmune strains of mice [9] and by a reduced SMLR in high-diabetes-incidence

NOD/Leiter (NOD/Lt) mice compared to the low-diabetes-incidence NOD/WEHI mice [10]. In humans, impairment of the equivalent autologous mixed lymphocyte reaction is associated with autoimmune disease including insulin-dependent diabetes [11].

During attempts to generate islet-reactive T-cell lines from the NOD islet lesion an autoreactive CD4⁺ T-cell line was isolated, providing an opportunity to further investigate a role for autoreactive T cells in the pathogenesis of insulin-dependent diabetes.

Materials and methods

Animals

NOD/WEHI (K^d, I-A^{g7}, I-E⁻, D^b) mice were bred under specific pathogen-free conditions and housed in the animal facilities of the Walter and Eliza Hall Institute of Medical Research. Their spontaneous incidence of diabetes at 180 days is less than 25%. At least

50% of 100-day-old female NOD mice become hyperglycaemic within 16 days after the i.p. administration of 300 mg/kg cyclophosphamide [12]. Female CBA mice (H-2^k) and D2GD mice (K^d, I-A^d, I-E⁻, D^b) were used as sources of control spleen cells.

Islet lymphocyte isolation

Whole islets with surrounding mononuclear cells ('insulitis') were isolated as described previously [13] from a 20-week-old female non-diabetic NOD/WEHI mouse. Briefly, the excised pancreas was digested in collagenase (Type XI; Sigma, St. Louis, Mo., USA), washed, and then purified by discontinuous density-gradient centrifugation through Ficoll (Ficoll 400, Sigma). Single islets with attached lymphocytes were hand-picked under a dissecting microscope and cultured at one islet per well in U-bottomed 96-well plates (Linbro; Flow Laboratories, McLean, Va., USA). T cells were cultured from these islets in RPMI-1640 plus 5% fetal calf serum (FCS) and propagated by the addition of 10 U/ml of recombinant interleukin-2 (IL-2) (a gift from Cetus Corporation, Emeryville, Calif., USA), and expanded every 2 weeks by stimulation with irradiated (3000 rad) spleen cells from 10-week-old female NOD/WEHI mice and 10 whole islets from 4-week-old female NOD/WEHI mice. The autoreactive T-cell line designated C2 was isolated from a single well. Cloning was performed by limiting dilution. Briefly, 0.5 cells per well were incubated with 5×10^4 irradiated (3000 rad) spleen cells from 10-week-old female NOD/WEHI mice plus 10 U/ml IL-2. After 14 days, wells (3 of 48) containing proliferating lymphocytes were expanded every 2 weeks by stimulation with irradiated NOD spleen cells and 10 U/ml IL-2 until sufficient cell numbers were available for analysis. The clones were designated C2.1, 2.2 and 2.3.

Proliferation assays

Cells of the C2 line (2×10^4) were incubated with irradiated (3000 rad) whole islets ($\times 5$) from 4-week-old female NOD/WEHI mice, 1×10^5 NOD WEHI spleen cells, CBA spleen cells or D2GD spleen cells in U-bottomed 96-well plates in 200 μ l RPMI-1640 plus 5% heat-inactivated FCS (Commonwealth Serum Laboratories, Parkville, Australia) and 5×10^{-5} mol/l 2-mercaptoethanol (2ME). Other islet antigen preparations included 0.1–10 μ g/ml sonicated NOD/WEHI islets, 0.1–1.0 $\times 10^4$ irradiated (3000 rad) trypsin-dispersed NOD/WEHI islet cells and 0.1–10 μ g/ml sonicated NIT cells ($a = n$ NOD beta-cell tumour line). Islet antigens were presented by irradiated NOD spleen cells. For antibody blocking experiments, antibodies recognizing NOD MHC I-A^{g7} [14] (OX-6; Serotec; Oxford, UK), NOD MHC K^d D^b (34.1.2S) or rat MHC class I (OX-18; Serotec) were added (final concentration 10 μ g/ml) with NOD spleen cells to C2. To exclude recognition by C2 of an exogenous peptide in FCS, C2 (5×10^4) were cultured in the presence of 0.5% heat-inactivated normal mouse serum and stimulated by syngeneic NOD spleen cells (2.5×10^5). On day 4, wells were pulsed with 1 μ Ci ³H-thymidine and harvested 9–16 h later for beta scintillation counting. Assays were performed in replicates of 3 to 6 and data expressed as the mean $\pm$ SD.

Fluorescence activated cell sorting (FACS)

Cells of the C2 line were stained for 30 min at 4°C with conjugated monoclonal antibodies to CD4 (L3T4 PE), CD8 (Lyt2 FITC) (Becton Dickinson, Mountain View, Calif., USA) or T-cell receptor (TCR) V β determinants including V β 8.1–2 antibody (KJ16) (provided by Dr. A. Bacelj, Walter and Eliza Hall Institute of Medical Research) followed by anti-rat Ig FITC (Kirkegaard and Perry Laboratories, Gaithersburg, Md., USA). Cells were washed and analysed by FACSCAN (Becton Dickinson).

Cytokine production

Cells (1×10^6) from the C2 line or C2.1, C2.2 or 2.3 clones were stimulated by irradiated (3000 rad) spleen cells (5×10^6) from female NOD/WEHI mice or anti-TCR (CD3) antibody (145-2C11,

provided by Dr. A. Kelso, Walter and Eliza Hall Institute of Medical Research) pre-coated at 5 μ g/ml onto wells of 24-well plates (Linbro; Flow Laboratories). At 24 h, supernatants were collected and stored at -70°C until bioassayed in serial dilution using the IL-2-dependent cytotoxic T-cell line (CTLL), interferon (IFN)- γ -sensitive WEHI-279, IL-6-dependent 7TDI, tumour necrosis factor (TNF)-dependent L929 and IL-4-dependent CT.4S cell lines. IL-10 was measured in an immunometric assay by Dr. J. Abrams (DNAX, Palo Alto, Calif., USA).

Adoptive transfer

Cyclophosphamide (300 mg/kg i.p.) was given to accelerate diabetes to 90–110-day-old female NOD/WEHI mice. The mice were divided into three treatment groups. Group A ($n = 28$) received 0.9% NaCl alone, group B ($n = 28$) 5×10^6 cells from the C2 line and group C ($n = 27$) 5×10^6 spleen cells from 4-week-old female NOD/WEHI mice i.v. on days 1, 4 and 7 following cyclophosphamide. Blood glucose was measured using BM 20–800 strips (Boehringer Mannheim, Sydney, Australia). Diabetes was diagnosed when blood glucose was greater than 15 mmol/l.

On day 16 pancreata were excised, fixed in Bouin's solution and sections stained with haematoxylin and eosin and Gomori aldehyde fuchsin. Insulitis was scored in the non-diabetic mice on a minimum of 15 islets per mouse, as follows: 0, no infiltrate; 1, peri-ductal infiltrate; 2, peri-islet infiltrate; 3, intra-islet infiltrate; 4, beta-cell destruction. The mean score for each pancreas was calculated by dividing the total score by the number of islets examined.

Cytotoxicity assays

The cytotoxic capability of the C2 line was measured against the murine mastocytoma cell line (P815) and lipopolysaccharide (LPS)-stimulated NOD/WEHI spleen cell blasts. LPS-blasts were prepared by culturing spleen cells (1×10^6 /ml) of 10-week-old female NOD/WEHI mice cultured in LPS (50 μ g/ml) for 48 h. Target cells were labelled with ⁵¹chromium (100 μ Ci/ 10^6 cells) for 90 min at 37°C, washed and resuspended in fresh medium. Cytotoxicity against the P815 cells was measured using a redirected lysis assay [15]. Anti-CD3 antibody (145-2C11) was added at 0.1 μ g/ml to ⁵¹chromium-labelled P815 cells to effect lysis mediated via the Fc receptors on P815 cells and the TCR on C2 cells. C2 were added in triplicate to P815 cells with or without added anti-CD3 antibody, or to LPS-blasts, at effector: target ratios of 0.6:1 to 150:1. After a 4 h incubation the supernatant was sampled and counted in a gamma counter and specific lysis calculated as:

$$\frac{\text{experimental} - \text{spontaneous cpm}}{\text{total} - \text{spontaneous cpm}}$$

Statistical analysis

Data were analysed by the chi-square test.

Results

C2 cells were stimulated by NOD/WEHI spleen cells but not by whole NOD/WEHI islets in the absence of added APC (Fig. 1A) or by allogeneic CBA or D2GD spleen cells (Fig. 1B). D2GD mice differ from NOD mice only at MHC class II I-A, which indicates that C2 recognizes I-A^{g7} $\pm$ peptide. The peptide does not appear to be derived from islets because whole NOD islets failed to stimulate C2. Other sources of islet antigen(s) including trypsin-dispersed single NOD islet cells, sonicated NOD islets and sonicated NIT cells, even when presented by NOD APC, failed to stimulate C2 cells more than NOD spleen cells alone (data not shown). The stimulation of C2 cells by

Fig. 1A,B. Response of C2 cells to **A)** irradiated NOD WEHI spleen cells or whole NOD WEHI islets, **B)** irradiated NOD WEHI, CBA or D2GD spleen cells (mean cpm $\pm$ SD, $n = 4$)

Fig. 2. Effect of antibodies against NOD MHC class II (OX-6) or class I (34.1.2S) on stimulation of C2 cells by NOD WEHI spleen cells (mean cpm $\pm$ SD, $n = 4$)

NOD/WEHI spleen cells was blocked at least 90% by a monoclonal antibody (OX-6) that recognizes I-A^{g7} [14], but not by an antibody (34.1.2S) against NOD MHC class I or a control antibody (OX-18) (Fig. 2). C2 cells did not recognize an exogenous peptide from FCS because

they still responded to NOD/WEHI spleen cells that had been cultured in normal mouse serum (Fig. 3).

By FACS analysis C2 cells were CD4⁺ and stained homogeneously with monoclonal antibody specific for the V β 8.1-2 TCR. They did not stain with antibodies against V β 2, V β 4, V β 5, V β 6, V β 7 or V β 11 (data not shown).

In adoptive transfer, C2 cells significantly suppressed the frequency of diabetes in NOD/WEHI mice given cyclophosphamide (21% vs 50%, $p < 0.05$); in contrast, young NOD/WEHI spleen cells did not have a significant effect on the frequency of diabetes (Table 1). Examination of pancreata from mice who had not become diabetic revealed no difference in the insulinitis scores between the groups (Table 1).

Table 2 summarizes the cytokine secretion profile of C2 cells. When stimulated with NOD/WEHI spleen cells, C2 cells secreted IFN- γ , TNF α/β and IL-6 but not IL-2, IL-4 or IL-10. The absence of IL-2 in the medium could reflect rapid consumption of IL-2 because C2 cells were capable of secreting IL-2 when stimulated by anti-CD3 antibody. IL-6 was secreted by irradiated spleen cells alone and the high levels of IL-6 measured in cultures containing C2 and NOD spleen cells presumably represent IL-6 production by both cell types. Although C2 cells stained homogeneously with antibody to TCR V β 8.1-2 they could nevertheless comprise distinct clones. This could explain their mixed cytokine profile. Limit dilution cloning of C2 cells yielded clones C2.1, 2.2 and 2.3. Cytokine secretion by C2.1, 2.2 and 2.3 clones is shown in Table 3. Each clone secreted IFN- γ and IL-6 in response to irradiated NOD spleen cells or anti-CD3 antibody. However, C2.2 appeared to be distinct from C2.1 and 2.3 in its capacity to secrete IL-2 in response to anti-CD3 antibody. The low levels of IL-4 were at the detection limit of the assay and could not be distinguished from zero. IL-10 was not detected in the supernatants of any of the C2 clones stimulated by irradiated NOD spleen cells or anti-CD3 antibody.

C2 cells were cytotoxic against P815 cells in the redirected lysis assay (Fig. 4). In the absence of added anti-CD3 antibody, C2 cells were unable to lyse P815 cells; in the presence of anti-CD3 antibody they displayed dose-dependent cytotoxicity, to a maximum of $34.1 \pm 7.7\%$ (mean $\pm$ SEM, $n = 3$) specific lysis at an effector: target ratio of 150:1. C2 cells were not able to lyse LPS-stimulated NOD spleen cells in the 4 h ⁵¹chromium release assay (data not shown).

Discussion

Islet-reactive T-cell clones isolated from the spleen and lymph nodes or islets of NOD mice can transfer insulinitis or diabetes [3-6], but the TCR α and β chains of NOD-derived islet-specific clones are heterogeneous [16, 17] indicating that a variety of T cells are involved in the process of beta-cell destruction. The existence of T-cell suppressor mechanisms in the NOD mouse is suggested by the effect of cyclophosphamide to accelerate the onset of diabetes [13] and the ability of spleen cells from non-diabetic NOD mice [18, 19] or T cells isolated from the NOD insulinitis lesion [6, 7] to prevent diabetes in adoptive transfer models. The in-

Fig. 3. Stimulation of C2 cells by irradiated NOD WEHI spleen cells in the presence of normal mouse serum (mean cpm $\pm$ SD, $n = 6$)

hibitory effect of the C2 line on cyclophosphamide-accelerated diabetes is further evidence that islets of non-diabetic NOD mice contain T cells which may suppress the destructive autoimmune process. It would appear that not only are effector T cells that cause beta-cell destruction heterogeneous but also T-cells with suppressor function, because diabetes has been prevented by the transfer of both CD4⁺ [7] and CD8⁺ [6] T cells. Furthermore, CD4⁺ T cells with suppressor function may express different V β TCR; the C2 line is V β 8.1–2, whereas the other CD4⁺ autoreactive line reported by Reich et al. [7] was V β 2.

Table 1. Frequency of diabetes and degree of insulinitis in cyclophosphamide (CP)-treated NOD/WEHI mice given either C2 cells (5×10^6) or young NOD/WEHI spleen cells (5×10^6) on days 1, 4, 7 and examined on days 10 and 16

	Day 10	Day 16	Insulinitis score ^a
Group A (CP only)	2/28 (7%)	14/28 (50%)	2.3 $\pm$ 1.1
Group B (CP + C2 cells)	0/28 (0%)	6/28 (21%) ^b	2.4 $\pm$ 0.9
Group C (CP + NOD WEHI spleen cells)	2/27 (7%)	11/27 (41%)	2.1 $\pm$ 0.9

^a Measured only in non-diabetic mice. ^b $p < 0.05$ vs Group A

Suppression of cyclophosphamide-induced diabetes in NOD mice by the C2 line confirms the earlier observation of Reich et al. [7] that autoreactive T-cells suppress the development of diabetes. The SMLR, due to recognition of self MHC class II by autoreactive CD4⁺ T-cells, is involved in the generation of immunoregulatory cells. Impaired suppressor T-cell function in NOD mice is suggested by their reduced SMLR compared to non-autoimmune strains of mice [9]. Furthermore, high-diabetes-incidence NOD/Lt mice have a reduced SMLR compared to low-diabetes-incidence NOD/WEHI mice [10]. Therefore, the difference in diabetes incidence between the lines may reflect a relative preservation of immunoregulatory cell function in NOD/WEHI mice. The isolation of the autoreactive C2 line from a non-diabetic NOD/WEHI mouse is consistent with this hypothesis.

C2 cells are autoreactive specifically against NOD I-A^{g7} because they are stimulated only by syngeneic spleen cells and this stimulation is blocked by a monoclonal antibody against the unique NOD MHC class II, I-A^{g7}. Whether autoreactive T cells recognize syngeneic MHC class II molecules in association with a specific peptide(s) is unknown. It has been suggested [20] that some autoreactive T cells recognize exogenous peptides derived from FCS. However, a requirement for FCS has been excluded here, and by others [21]. T cells from unprimed BALB/c mice have been reported to react to syngeneic MHC class II peptides (I-A α and I-A β) [22] and one of the peptides eluted from purified class II I-A^b molecules was from the α chain of the class II molecule I-E^b [23]. Of possible relevance therefore is the finding that a synthetic peptide spanning residues 48–60 of NOD I-A β stimulated NOD T cells [24]. This peptide contains a histidine at position 56 and serine at position 57, unique to the NOD mouse [25]. This implies that C2 might recognize an I-A^{g7} peptide presented in the context of I-A^{g7} itself. However, in experiments using synthetic peptides matching residues 1–14, 48–60 and 62–78 of the NOD class II I-A β chain, kindly provided by Dr. B. Singh, Department of Immunology, University of Alberta, Alberta, Canada, we have observed no stimulation of C2 cells by irradiated APC pre-pulsed with peptides or by glutaraldehyde-fixed APC in the presence of peptides (data not shown).

CD4⁺ T cells have been broadly classified into Th₁ and Th₂ [26]. Th₁ cells secrete IL-2 and IFN- γ and preferentially induce macrophage activation and delayed type hypersensitivity, whereas Th₂ cells secrete IL-4, 5, 6 and 10 that

Table 2. Cytokine secretion in vitro at 24 h by the C2 cell line stimulated by NOD spleen cells or anti-CD3 antibody (145-2C11)

	IFN- γ (U/ml)	IL-6 (U/ml)	IL-2 (U/ml)	TNF α/β (pg/ml)	IL-4 (U/ml)	IL-10 (pg/ml)
C2 cells	< 0.04	< 3	0.28	< 2.5	< 0.13	< 20
NOD spleen cells	< 0.04	51	< 0.04	< 2.5	< 0.13	< 20
C2 cells + NOD spleen cells	3402	4747	< 0.04	2500	–	< 20
C2 cells + anti-CD3 antibody	6557	408	3.2	> 5000	< 0.13	< 20

IL, Interleukin; TNF, tumour necrosis factor; IFN, interferon

Table 3. Cytokine secretion in vitro by C2.1, 2.2 and 2.3 clones stimulated by NOD WEHI spleen cells (APC) or anti-CD3 antibody (145-2C11)

	IFN- γ (U/ml)	IL-6 (U/ml)	IL-2 (U/ml)	IL-4 (U/ml)	IL-10 (pg/ml)
C2.1 + anti-CD3	1371	3.7	<0.01	0.22	<20
C2.1 + APC	605	179	<0.1	<0.1	<20
C2.1 alone	0.6	<0.3	<0.1	<0.1	<20
C2.2 + anti-CD3	77,419	167	4.4	<0.1	<20
C2.2 + APC	597	125	<0.1	<0.1	<20
C2.2 alone	0.4	<0.3	<0.1	<0.1	<20
C2.3 + anti-CD3	1242	3.2	<0.1	0.12	<20
C2.3 + APC	140	1.6	<0.1	<0.1	<20
C2.3 alone	<0.2	<0.3	<0.1	<0.1	<20
APC alone	0.4	0.88	<0.1	<0.1	<20
Medium	<0.2	<0.3	<0.1	<0.1	<20

IL, Interleukin; IFN, interferon

Fig. 4. Specific lysis (mean $\pm$ SEM, $n = 3$) by C2 cells of P815 cells in the presence $\square$ or absence $\blacksquare$ of anti-CD3 antibody (145-2C11). E:T, Effector:target

provide help for B-cell production of high titre antibodies. Although C2 cells stained uniformly with antibody against V β 8.1–2 TCR and C2 clones produced both IFN- γ and IL-6, the finding that clone C2.2 produced IL-2 and clones C2.1 and C2.3 did not suggests that the cytokine profile of the C2 line was due to a mixture of at least two distinct clones. They are typical of neither Th₁ nor Th₂, although IFN- γ secretion, with a lack of IL-4 and IL-10 secretion, is more characteristic of Th₁. Whether the difference in cytokine production between the clones alters the effect on cyclophosphamide-induced diabetes has not been assessed.

Studies in vitro have demonstrated that autoreactive T cells are functionally heterogeneous and may display suppressor or helper properties or both. In humans, autoreactive T-cell clones inhibit the proliferation of autologous T cells stimulated with antigens or mitogens [27]. However, autoreactive T-cell clones, activated by syngeneic B cells, induce immunoglobulin (Ig) production by syngeneic [28–30] or allogeneic [28, 31] B cells. This B-cell help appears to be mediated by soluble factor(s) because the supernatant derived from autoreactive T-cell clones activated by

syngeneic B cells induces Ig production by B cells [28]. Paradoxically, the same autoreactive T-cell clones may suppress Ig production from pokeweed mitogen-stimulated syngeneic B cells [28, 32]. The functional effects of some autoreactive T-cell clones may therefore be determined by the state of B-cell activation.

In what ways could autoreactive T cells modify autoimmunity in the NOD mouse? The isolation of the C2 line from the insulinitis lesion suggests that C2 cells might recognize an islet peptide in association with I-A^{g7}. However, the inability of NOD islets to augment the response of C2 cells to NOD spleen cells argues against this. Alternatively, C2 cells in the islets could be generated as part of the inflammatory process, e.g. after activation of macrophages and the up-regulation of I-A. Macrophages are the first cells to infiltrate NOD islets [33] and are necessary for beta-cell destruction [34], presumably by presenting beta-cell specific peptides to cytotoxic CD4⁺ or CD8⁺ T cells. Autoreactive T cells could inhibit the function of activated macrophages by the release of cytokines or by direct cytotoxicity, or both. There is also evidence that some murine T cells express functional MHC class II [35, 36], in which case it is possible that C2 could act directly on islet-specific T cells, although at this stage we have not determined whether injected C2 cells traffic to islets. TNF is one of the cytokines secreted by C2 cells following stimulation by syngeneic antigen presenting cells. Systemic administration of TNF- α to NOD mice reduces their incidence of diabetes [37, 38] and TNF- α together with antigen may induce antigen-specific tolerance [39]. Thus, it is conceivable that intra-islet release of TNF could be immunosuppressive. C2 cells do not appear to act via the secretion of IL-10, which inhibits delayed type hypersensitivity reactions [40] typified by the insulinitis lesion. Some CD4⁺ T cells are capable of cytotoxicity which appears to be mediated through a TCR interaction that results in DNA fragmentation and apoptotic cell death [41, 42]. Autoreactive T-cell lines are capable of lysing syngeneic LPS-blasts [30]. C2 cells were cytotoxic as measured by redirected lysis, although they did not lyse LPS-stimulated spleen cell blasts. A cytotoxic effect on APC cannot be excluded, however, because CD4⁺-mediated cytotoxicity could be delayed [42] and might not be detected by a short-term in vitro test system. Although C2-mediated cytotoxicity was dependent on stimulation of the TCR by anti-CD3 antibody, a role for cytokines in target cell lysis cannot be excluded.

In conclusion, the present study provides further evidence in the NOD mouse for regulatory T cells which are autoreactive against self-class II MHC and supports the view that beta-cell destruction represents an imbalance between cytotoxic and regulatory T cells.

Acknowledgements. We thank Ms. P. Ford and Dr. A. Kelso for performing the IL-2, IL-4, IL-6 and IFN- γ assays Dr. J. Abrams of DNAX for performing the IL-10 assay and Dr. W. Heath for technical advice.

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Received: 21 December 1992
and in revised form: 23 March 1993

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