Diabetologia

Destruction of conditional insulinoma cell lines in NOD mice: A role for autoimmunity

P. Cattan[1], D. Rottembourg[1], S. Cottet[2], I. Tardivel[1], P. Dupraz[2], B. Thorens[2], C. Boitard[3], J. C. Carel[4]

[1] INSERM U561, Groupe hospitalier Cochin-Saint Vincent de Paul, Paris, France
[2] Institute of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland
[3] INSERM U561 and Department of Clinical Immunology, Groupe hospitalier Cochin-Saint Vincent de Paul, Université Paris V, Paris, France
[4] INSERM U561 and Department of Paediatric Endocrinology, Groupe hospitalier Cochin-Saint Vincent de Paul and Faculté Cochin, Université Paris V, Paris, France

Abstract

Aims/Hypothesis. βTC-tet (H2^k) is a conditional insulinoma cell line derived from transgenic mice expressing a tetracycline-regulated oncogene. Transgenic expression of several proteins implicated in the apoptotic pathways increase the resistance of βTC-tet cells in vitro. We tested in vivo the sensitivity of the cells to rejection and the protective effect of genetic alterations in NOD mice.
Methods. βTC-tet cells and genetically engineered lines expressing Bcl-2 (CDM3D), a dominant negative mutant of MyD88 or SOCS-1 were transplanted in diabetic female NOD mice or in male NOD mice with diabetes induced by high-dose streptozotocin. Survival of functional cell grafts in NOD-scid mice was also analyzed after transfer of splenocytes from diabetic NOD mice. Autoreactive T-cell hybridomas and splenocytes from diabetic NOD mice were stimulated by βTC-tet cells.
Results. βTC-tet cells and genetically engineered cell lines were all similarly rejected in diabetic NOD mice and in NOD-scid mice after splenocyte transfer. In 3- to 6-week-old male NOD mice treated with high-dose streptozotocin, the cells temporarily survived, in contrast with C57BL/6 mice treated with high-dose streptozotocin (indefinite survival) and untreated 3- to 6-week-old male NOD mice (rejection). The protective effect of high-dose streptozotocin was lost in older male NOD mice. βTC-tet cells did not stimulate autoreactive T-cell hybridomas, but induced IL-2 secretion by splenocytes from diabetic NOD mice.
Conclusion/Interpretation. The autoimmune process seems to play an important role in the destruction of βTC-tet cells in NOD mice. Genetic manipulations intended at increasing the resistance of beta cells were inefficient. Similar approaches should be tested in vivo as well as in vitro. High dose streptozotocin influences immune rejection and should be used with caution. [Diabetologia (2003) 46:504–510]

Keywords βTC-tet cell line, autoimmunity, NOD mice, NOD-scid, Bcl-2, MyD88, SOCS-1, streptozotocin.

Tumoural beta-cell lines represent an useful tool for experimental studies of Type 1 diabetes mellitus and could be a candidate for replacement cell therapy in diabetic patients, if cellular growth is tightly controlled. βTC-tet is an insulinoma cell line originating from a double transgenic C3H (H2^k) mouse in which the expression of the SV40-T Ag is under control of a tetracyclin operator-transactivator system [1]. Oncogen expression is reversibly switched off in vitro and in vivo by tetracyclin in the culture medium or in drinking water. When transplanted in syngenic or allogenic mice made diabetic with a high dose i.v. streptozotocin (STZ), these cells durably reverse hyperglycaemia in 2 to 4 weeks [2, 3]. Administration of tetracyclin to recipient mice prevents the development of massive tumours and death from hypoglycaemia. The lack of allogenic grafts rejection in this setting has been ascribed to the immunosuppressive effect of STZ

Corresponding author: J. C. Carel, INSERM U561, Groupe hospitalier Cochin-Saint Vincent de Paul, 82 av Denfert Rochereau, 75014 Paris, France
E-mail: carel@cochin.inserm.fr
Abbreviations: STZ, treprozotocin; NOD, on obese diabetic; scid, evere combined immunodeficiency.

[3]. The survival of βTC-tet cells in an autoimmune environment has not been evaluated. βTC-tet cells can easily be transduced using lentivirus vectors, allowing the overexpression of molecules that could potentially increase the resistance of these cells to noxious stimuli. Indeed, overexpression of Bcl-2 decreases cytokine and hypoxia induced cell death [2]. Moreover, transduction of CDM3D cells with a dominant negative mutant of MyD88, an adaptor protein linking the IL-1 receptor to downstream signalling molecules, blocks the apoptotic signals induced by IL-1R engagement [4]. In addition, transduction of CDM3D cells with SOCS-1, a protein that prevents JAK/STAT-dependent inhibition of insulin transcription and secretion in response to IFN-γ, prevents beta-cell function impairment and death induced by IFN-γ [5]. However, the protective effect of these genetic alterations was only tested in vitro. The aims of this study were firstly, to analyze the sensitivity of βTC-tet cells to autoimmune rejection in the NOD mouse model and secondly, to test the resistance of genetically engineered βTC-tet cell lines in an autoimmune environment.

Materials and Methods

Cell lines. Parental βTC-tet cells [1] and three genetically engineered lines using lentiviral vectors were used. CDM3D cells were obtained by overexpression of the anti-apoptotic gene Bcl-2 [2]. The CDM3D cell line was additionally transduced to induce the expression of the dominant negative mutant of MyD88 (MyD88lpr cell line; [4]), or the over-expression of SOCS-1 (SOCS cell line; [5]). Cells were maintained in culture in Dulbecco's modified Eagles medium (DMEM), supplemented with horse serum (15%), foetal calf serum (7.5%), penicillin-streptomycin (1%), MEM 100 mM (1%), L-Glutamine (1%), at 37°C, CO_2 5%. Before in vitro and in vivo experiments, cells were harvested after a 2-min incubation in trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA) and were washed twice in DMEM.

Stimulation of diabetic NOD splenocytes. An Elispot assay was used to evaluate the stimulation of diabetic NOD splenocytes and their IL-2, IL-4 and IFNγ secretion profiles in response to βTC-tet cells [6]. We coated 96-well nitrocellulose plates (Millipore, St Quentin en Yvelines, France, MAHA S45) with relevant antibodies (anti-IL-2, Pharmingen, BD Bioscience, Le Pont de Claix, France, 18161D; anti-IL-4, Pharmingen 18191D; anti-IFN-γ, Pharmingen 18181D) at 10 μg/ml in PBS buffer at 37°C overnight. Plates were washed in PBS buffer, saturated with DMEM 10% SVF, and incubated with $5×10^5$ NOD splenocytes and 10^5 βTC-tet cells or 5.10^4 NOD islet cells for 48 h at 37°C. Plates were washed twice in PBS-Tween 0.05% and incubated 2 h at 37°C with detection biotin-coupled antibodies (anti-IL2, Pharmingen 18172D; anti-IL-4, Pharmingen 18042D; anti-IFN-γ, Pharmingen 18112D). Plates were washed twice in PBS-Tween and incubated for 1 h with extravidine-PA (Sigma Aldrich, l'Isle d'Abeau Chesnes, France, E2636) diluted 1/6000 in PBS-Tween BSA and stained with extravidine substrate (Bio-Rad, Ivry sur Seine, France, kit 170–6432). Spots were read using a Zeiss automated microscope and KS Elispot software (C. Zeiss, Le Pecq, France).

Stimulation of beta cell autoreactive T-cell hybridomas. The following T cell hybridomas were used: BDC2.5 [7]; 12.20 generated by fusion of islet infiltrating T cells with α–β-BW7.136-CD4+ lymphoma, reactive with an undefined islet antigen, with similarities to BDC2.5; LTI27–41, IA^{g7} restricted and reactive to preproinsulin II26–41 peptide [8]. 10^5 hybridoma cells, $5×10^5$ irradiated NOD spleen cells as antigen presenting cells and $2×10^4$ βTC-tet cells as antigen source, were cultured overnight. βTC-tet cells were used after standard culture, after tetracyclin growth-arrest, as lysates (sonication, freeze-thawing) or after explantation from a functioning graft. $2×10^4$ irradiated NOD or C3H islet cells were used as controls. Culture supernatants were assayed for IL-2 using the CTLL cell line.

Mice. NOD mice ($H2K^dD^bIA^{g7}$) were bred in our facilities under specific pathogen-free conditions and checked every 6 months for bacterial, viral and parasitic infections. The spontaneous incidence of diabetes in this colony is 75% for female and 40% for male. NOD-scid mice were obtained from INSERM U375, Villejuif, France, C3H mice ($H2^k$) and C57BL/6 ($H2^b$) mice from CERJ, Le Genest Saint-Isle, France. All animal manipulations were conducted and monitored under protocols reviewed and approved by our local animal ethic committee.

Transplantation in immunocompetent mice. Clusters of $2×10^6$ βTC-tet cells, formed during a 48-h incubation on a rotary motion system, were transplanted under the left kidney capsule of mice [9]. In some experiments, animals were made diabetic by a single i.v.-injection, 3 days before transplantation, of STZ (200 mg/kg; Sigma-Aldrich) reconstituted in a citrate/citric acid buffer (0.01 M, pH 4.5). Glycaemia was measured with an Euroflash haemoglucometer (Lifescan, Issy les Moulineaux, France). Diabetes was defined by a persistent glycaemia greater than 350 mg/dl. Before transplantation, animals were anaesthesized with avertin (375 mg/kg i.p.; 2.2.2-Tribromo-methanol; Sigma-Aldrich). After transplantation, glycaemia was measured twice a week. In the case of hyperglycaemia, animals received 2 to 3 units of insulin s.c. (Ultratard, 100 UI/ml; Novo Nordisk, Boulogne Billancourt, France). When glycaemia fell under 150 mg/dl, tetracycline (1 g/l, Sigma-Aldrich) was added to the drinking water in order to stop tumoural growth. Transplant function was defined by normoglycaemia in diabetic recipients or by hypoglycaemia in normoglycaemic recipients. Transplant survival was assessed by histological examination of transplanted kidneys at 6 weeks post-transplant.

Transplantation in NOD-scid (severe combined immunodeficiency) mice. In that model, NOD-scid mice were rendered diabetic by STZ (200 mg/kg) and transplanted with βTC-tet cells. When normoglycaemia was observed, tetracyclin was added to the drinking water. After 2 to 7 days, $2×10^7$ diabetic NOD splenocytes (obtained from a pool of two to three mice) were transferred i.v. T lymphocyte transfer was controlled by FACS-detection of CD3 positive cells in the peripheral blood using a hamster monoclonal antibody to mouse CD3 (Cedarlane, Tebu, Le Perray en Yvelines, France). Unmanipulated NOD-scid mice were used as controls for the incidence of diabetes after splenocyte transfer (two to three controls and two to four experimental animals for each transfer experiment using the same pools of diabetogenic cells).

Histology. We stained 5-μm-thick sections of paraffin embedded transplanted kidney with hematoxilin-eosin. Additionally, Bcl-2 immunohistochemical staining was carried out with mouse anti-human Bcl2 monoclonal antibody (Ancell,

Table 1. Function and survival of βTC-tet grafts in different types of recipients.

Type of recipient	Streptozotocin treatment	Frequency of functional grafts, n (%)	Frequency of functional grafts at sacrifice or ≥6 weeks post-transplant, n (%)
C3H	+	29/33 (88%)	29/33 (88%)*
C3H	−**	7/7 (100%)	7/7(100%)*
C57BL/6	+	18/20 (90%)	18/20 (90%)*
C57BL/6	−**	0/10 (0%)	0/10 (0%)
Diabetic female NOD	−	0/54 (0%)	0/54 (0%)
3–6 wk-old male NOD	+	15/28 (54%)	3/28 (11%)
3–6 wk-old male NOD	−**	1/26 (4%)	1/26 (4%)
7–12 wk-old male NOD	+	1/15 (7%)	1/15 (7%)
7–12 wk-old male NOD	−**	0/2 (0%)	0/2 (0%)

Results obtained with parental and genetically modified βTC cells were similar and were therefore pooled.
* prolonged graft function was observed in three mice in each of these groups, kept alive for 100 days.

** when non diabetic recipients were used, hypoglycaemia was used as a readout of transplant function and histology was used to confirm graft implantation

Bayport, Minn., USA), streptavidine-PA and Fast red (Dako, Trappes, France).

Results

βTC-tet cells are rejected in diabetic NOD mice. Transplantation of parental βTC-tet cell clusters did not reverse hyperglycaemia in any of 12 spontaneously diabetic female NOD mice. Similarly, transducted βTC-tet cells, overexpressing either Bcl-2 alone (CDM3D, *n*=12), or in combination with a dominant negative mutant of MyD88 (MyD88lpr, *n*=17) or with SOCS-1 (SOCS, *n*=13) did not revert hyperglycaemia (Table 1). Sequential histological analysis of graft bearing kidneys was carried out on a subset of mice (Fig. 1). Seven days after implantation, the grafted cells were present and infiltrated by mononuclear cells. Fourteen days after implantation, the cells were massively infiltrated and 21 days after implantation, the cells were no longer detectable and replaced by a scar. At the time of death, no residual graft was detected in 46 of 50 mice followed for 6 weeks. However, in four cases, a voluminous infiltrated tumour was detected, although no in vivo function was observed.

In order to ascertain the functionality of βTC-tet cells in vivo, all cell lines used were transplanted in parallel to syngenic (C3H) untreated and STZ-diabetic mice (Table 1). Hyperglycaemia was reverted in 3 to 4 weeks and graft function persisted until killing (up to 100 days). Nephrectomy induced a return to hyperglycaemia in streptozotocin-treated mice. Histological analysis of transplanted kidneys showed a graft without any cellular infiltrate (Fig. 1). Additionally, Bcl-2 expression was detected in the graft several weeks after syngenic transplantation of genetically engineered cell lines. We observed no difference in terms of in vivo function and control of tumoural growth between parental and modified cell lines.
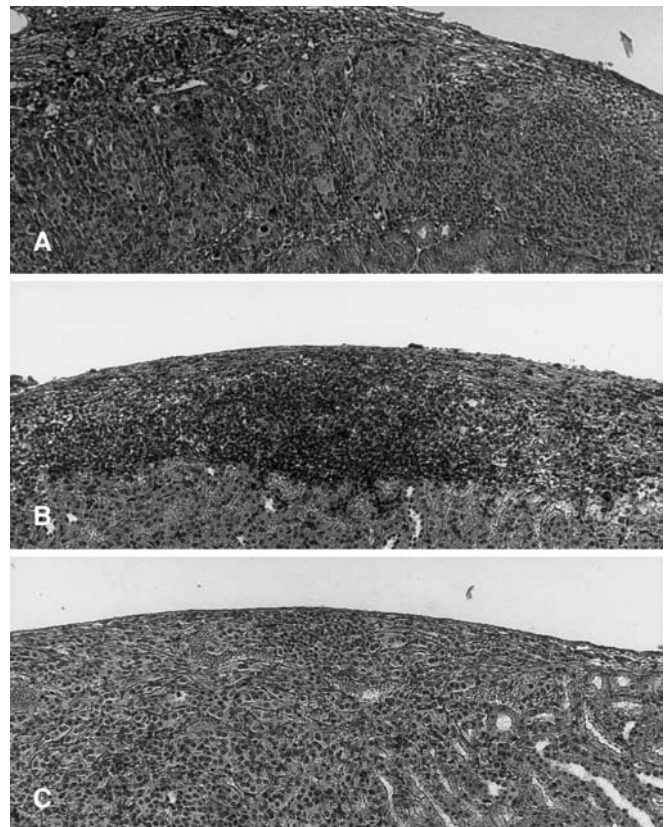

Fig. 1A–C. Sequential histological analysis of βTC-tet graft bearing kidneys from female diabetic NOD mice. Clusters of 2 10⁶ βTC-tet cells were transplanted under the left kidney capsule of NOD mice and histological analysis was carried out at day 7 post-transplant (**A**) and day 14 post transplant (**B**); (**C**) the aspect of a similar graft in a syngenic recipient; 5-μm-thick sections of paraffin embedded transplanted kidney were stained with haematoxilin-eosin; magnification : ×100

βTC-tet cells survive indefinitely in STZ-diabetic C57BL/6 mice but are rejected in untreated C57BL/6 mice. In order to analyze βTC-tet cell survival in an allogenic non autoimmune environment, and to con-
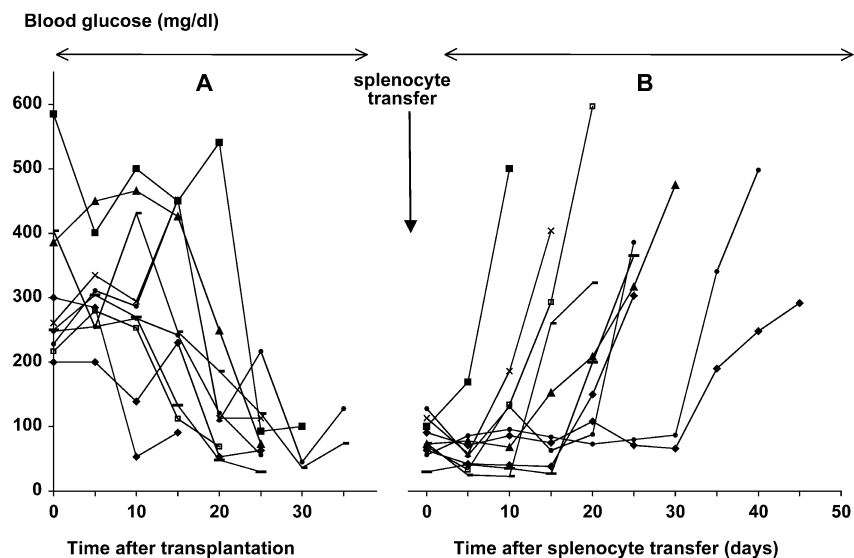
Fig. 2A, B. Blood glucose concentrations in NOD-scid mice transplanted with cells lines and transferred with diabetogenic splenocytes. (**A**) Clusters of 2×10^6 βTC-tet cell lines were transplanted in NOD-scid mice with streptozotocin induced diabetes; when normoglycaemia was obtained (**B**), 2×10^7 diabetic-NOD splenocytes were injected i.v. (arrow); hyperglycaemia indicates graft rejection

Fig. 3. Outcome after splenocyte transfer in the NOD-scid model. In βTC-tet transplanted animals, the reoccurrence of diabetes due to βTC-tet cells rejection ($n=10$, ●—), or death from hypoglycaemia ($n=11$, ■—) are shown. In naive NOD-scid mice, the occurence of diabetes is shown ($n=11$, ○—). The same pools of diabetogenic spleen cells were used in transplanted and control mice

firm the protective effect of STZ in that setting [3], βTC-tet cell lines were transplanted in untreated and in STZ-diabetic C57BL/6 mice. In untreated recipients, no βTC-tet line function (Bcl-2 in combination with MyD88lpr, $n=5$ or Bcl-2 in combination with SOCS-1, $n=5$) was observed and histological analysis of transplanted kidneys done 3 weeks after transplantation showed a complete disappearance of the graft. Conversely, 18 of 20 STZ-diabetic C57BL/6 mice showed a graft function, a median of 21 days (13 to 52 days) after transplantation (Table 1). No loss of function was observed until killing at 6 weeks after transplant or under longer follow-up in three mice (100 days). Histological analysis of transplanted kidneys at the time of killing showed a mild cellular infiltrate.

βTC-tet cells are rejected in the NOD-scid mice model. Given the slow kinetics of implantation of βTC-tet cell lines in vivo (3 to 4 weeks), it was difficult to differentiate primary graft failure from rejection in diabetic NOD mice. Therefore, the NOD-scid mice model was designed to allow implantation of cells in a permissive environment, followed by tetracycline treatment to avoid tumour progression and hypoglycaemia, and then by transfer of autoreactive lymphocytes. The experiment was carried out with all four types of βTC-tet cell lines (βTC-tet, $n=2$; CDM3D, $n=8$; MyD88lpr, $n=9$; SOCS-1, $n=5$), but since a different pattern was not observed, the results were combined. After transplantation of βTC-tet cell lines in STZ-diabetic NOD-scid mice, the median delay to normoglycaemia was 25 days (11 to 36 days). After transfer
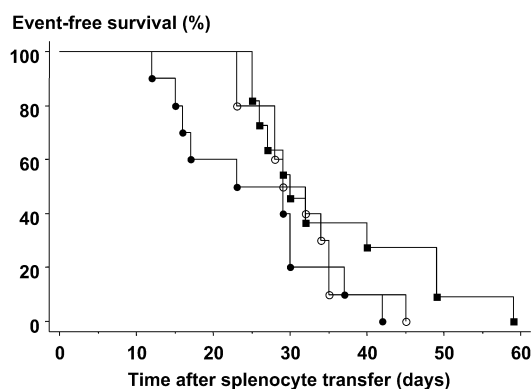
of diabetogenic splenocytes in 24 mice, diabetes recurred in 10 mice after a median of 26 days (12 to 42 days; Figs. 2 and 3). Among the remaining 14 mice, 11 died in a context of hypoglycaemia, a median of 30 days (25 to 59 days) after transfer and three were killed between day 35 and day 42 post transfer for histological analysis. In parallel, naive NOD-scid mice transferred with diabetogenic splenocytes became diabetic after a median of 30.5 days (23 to 45 days). Event-free survival was similar in the three groups of mice by the Log-Rank test (Fig. 3). Histological analysis of transplanted kidney showed a tumour in all cases, although of variable size, with cellular infiltrate. There was no difference in histology between mice with loss of graft function and those who died from hypoglycaemia. In control mice, islets were massively infiltrated at time of diabetes.

βTC-tet cells survive temporarily in young STZ-diabetic NOD mice but not in older mice. We then evalu-
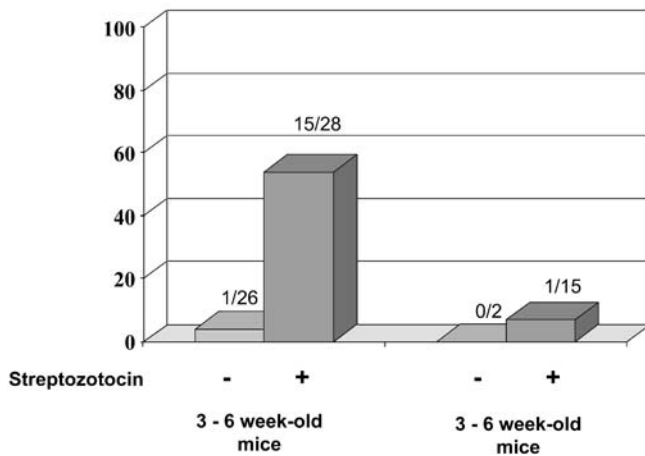
Graft survival (%)

Fig. 4. Outcome of βTC-tet cell grafts in male NOD mice of various ages, untreated or treated with high dose streptozotocin (200 mg/kg i.v.). Clusters of 2.10⁶ βTc-tet cells were transplanted in STZ-diabetic and naive male NOD mice of different ages (3–6-wk-old or 7–12-wk-old)
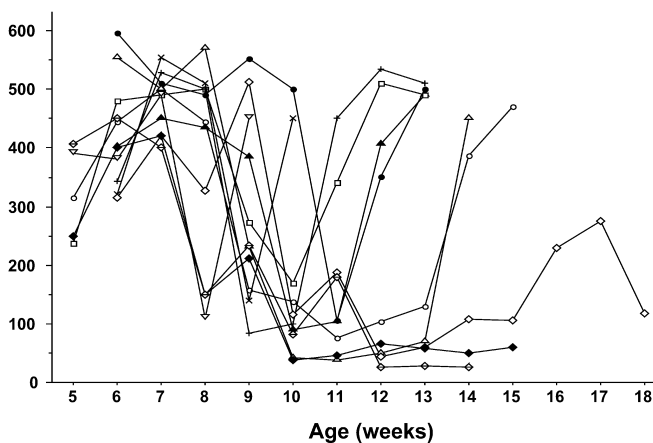
Blood glucose (mg/dl)

Fig. 5. Blood glucose kinetics in 11 young STZ-diabetic male NOD mice with graft function and long-term follow-up. Clusters of 2.10⁶ βTc-tet cells were transplanted in STZ-diabetic 5–6-wk-old male NOD mice

Number of spots
(n/5x10⁵ spleen cells)

Fig. 6. Quantitative Elispot analysis of IL-2 secretion profile by splenocytes from diabetic NOD mice after stimulation by NOD islet cells and βTC-tet (CDM3D) cells (the means ± SD of triplicates is shown; four experiments are shown)

[10] and we suspected that induction of diabetes with this agent might have increased the incidence of beta-cell destruction in young male NOD mice. Therefore, we carried out the same experiment using naive 4- to 6-week-old male NOD mice. Surprisingly, none of 26 mice but one showed graft function (e.g. hypoglycae-mia; Fig. 4). When the grafts were examined 6 weeks after transplantation, no tumour could be detected in these 25 mice. Therefore, it was concluded that STZ had prevented the early rejection phase in young male NOD mice. We then examined if the same protective effect of STZ would be observed in older mice. In 7- to 12-week-old STZ-treated male NOD mice, 14 out of 15 grafts were rejected before establishment of graft function. As expected, none of the two grafts carried out in the 7- to 12-week-old naive male NOD mice survived. Therefore, the permissive effect on ßTC-tet cell engraftment of STZ was only observed in 3- to 6-week-old male NOD mice.

βTC-tet cell do not stimulate anti-islet T-cell hybrid-omas but stimulate diabetic NOD splenocytes to secrete IL-2. To evaluate if the βTC-tet cells could be recognized by the NOD autoimmune process, we evaluated if these cells could stimulate diabetic NOD splenocytes. βTC-tet cells stimulated splenocyte IL-2 secretion similarly to NOD islets in three of four experiments (Fig. 6). No effect on IL-4 or IFNγ secretion was observed. By contrast, the three NOD T cell hybridomas, while stimulated by NOD and to a lesser extent by C3H islet cells, were not activated by the βTC-tet cells lines. Various cell preparations were also unable to stimulate these hybridomas: after tetracyclin-induced growth arrest, sonication, freeze-thawing or after explantation from a NOD-scid mouse.

ated whether βTC-tet cells would survive in NOD mice rendered diabetic by high dose STZ before the activation of islet autoimmunity. Transplantation of βTC-tet cell lines reversed diabetes in 15 of 28 STZ-diabetic 3- to 6-week-old male NOD mice in a median of 26 days (21 to 35 days, Fig. 4). Upon longer follow-up in 11 of the 15 mice with functioning graft, diabetes recurred in eight mice at a median age of 11 weeks (9 to 14 weeks; Fig. 5) or a median of 42 days (28 to 63 days) after transplantation. Histological analysis at rejection showed grafts of various size with cellular infiltrate.

Such a rate of beta-cell destruction in the male NOD background was not expected. STZ has been suspected to induce autoimmunity in animal models
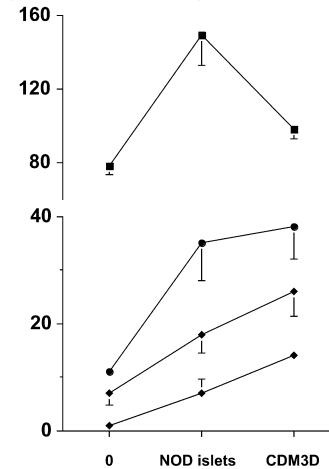
Discussion

We have studied the outcome of a mouse conditional insulinoma cell line after implantation in NOD mice. Our principal findings are as follows: (i) βTC-tet are rejected in autoimmune NOD mice; (ii) genetic manipulations that have been shown in vitro to increase resistance to apoptotic stimuli did not protect the cells from destruction; (iii) when transplanted in young male NOD mice, before the installation of the autoimmune process, streptozotocin induced a temporary protection from rejection of the cell line. The exact mechanisms by which βTC-tet lines are destroyed by NOD lymphocytes deserves discussion and more specifically, it is of interest to discuss whether autoimmune or allogenic mechanisms were involved, since βTC-tet cells display both beta-cell specific antigens and allogenic (H2k) antigens. In view of MHC restriction, cells involved in the autoimmune reaction cannot kill allogenic (H2k) cells by the CD8-mediated direct pathway, but can do so by the indirect modes of cellular insult (mediated by Fas/FasL interactions or by cytokine toxicity). Several lines of argument strongly suggest that the autoimmune process of the NOD mouse is involved in βTC-tet cells rejection. Firstly, STZ differentially affected βTC-tet cell rejection in a pure allogenic or autoimmune model. As described by others [3], a high dose of STZ had a long-term protective effect on βTC-tet cell rejection by allogenic C57BL/6 mice. When the same experiment was carried out using young (3- to 6-week-old) NOD mice, a similar protective effect of STZ was observed. In contrast, when older (7- to 12-week-old) NOD mice were used as recipients, STZ was ineffective to prevent graft rejection. Studies on the natural history of autoimmune diabetes in NOD mice have shown that autoreactivity begins after the age of 3 to 4 weeks, although diabetes occurs around 4 to 6 months [11]. Therefore, when STZ is used in "pre-autoimmune" 3- to 6-week-old NOD mice, it can prevent rejection in a pattern similarly to what is observed in the pure allogenic situation. In contrast, when the autoimmune process is ongoing, STZ can no longer prevent graft destruction.

One further argument can be derived from the long term outcome of accepted grafts in C57BL/6 or NOD mice. βTC-tet cells survive indefinitely in STZ treated C57BL/6 mice [3], whereas in young STZ-treated male NOD mice, the grafts are secondarily rejected at a median age of 11 weeks. Here again, the differential pattern in C57BL/6 compared with NOD mice suggests the involvement of autoimmunity. One drawback to this argument is that autoimmune diabetes occurs at a low frequency in male NOD mice, and generally after the age of 12 weeks. Therefore synergistic mechanisms between autoimmunity and allogenic rejection might be involved.

The third argument for a role of autoimmune compared with allogenic destruction of βTC-tet cells comes from the similar destruction kinetics of transplanted cells compared with endogenous islets in the NOD-scid model (26 vs. 30.5 days). However, in this model, death of about 50% of mice from hypoglycaemia, complicates the interpretation of the findings. This reflects an incomplete graft destruction at the time of death while histological analysis showed a major cellular infiltrate attesting to an ongoing rejection phenomenon. One possible explanation is that the large ßTC-tet mass could have overcome the rejection capacities of transferred NOD splenocytes. We consider death from hypoglycaemia as a limitation of the NOD-scid model rather than an indication of βTC-tet cell resistance to the immune system.

Further, we studied in vitro if βTC-tet cells were recognized by the NOD autoimmune process. βTC-tet cells stimulated IL-2 secretion by diabetic NOD splenocytes similarly to NOD islets, suggesting that βTC-tet cells were recognized by autoimmune lymphocytes although alloreactivity could also be involved. In contrast, βTC-tet failed to activate auto-reactive I-A^{g7}-restricted hybridomas directed to insulin or to unknown antigens (BDC2.5 and 12.20) whereas C3H islets were stimulated the hybridomas, in the presence of NOD (I-A^{g7}) splenocytes . Whether this difference is due to antigen disposal of tumour cells by NOD antigen presenting cells or to lack of expression of antigens themselves (BDC2.5 and 12.20) could be evaluated in vivo through transfer experiments using TCR transgenic mice as donors. Several mediators of beta-cell death are suspected to be involved in the destruction of syngenic islets in NOD mice : IL-1 β, TNF α, IFN γ, FasL. Over-expression of protective genes like Bcl-2 [2, 12, 13], A20 [14], or blockade of cytokine signalling [4, 5, 15] protect from cytokine induced beta-cell death in vitro. Most of these genetic manipulations have either been shown to be ineffective in vivo (Bcl-2) [16] or have not been evaluated in vivo [17]. Our results underline the importance of testing such strategies in vivo. Several reasons could be advanced for the lack of protection from allogenic or autoimmune rejection in vivo. Firstly, multiple apoptotic pathways are probably involved simultaneously. Blocking one of them would not be sufficient, and a combination of molecular strategies should be tested for the prevention of beta-cell destruction. Secondly, transgene expression levels could be insufficient to confer resistance in vivo. However expression of transgenes in βTC-tet cell lines is stable after years of culture, and Bcl-2 was expressed in vivo, weeks after transplantation.

In conclusion, the βTC-tet conditional insulinoma cell line does not survive in the NOD environment. Several lines of evidence suggest that the autoimmune process plays a major part in cellular destruction. Genetic manipulations of beta cells aimed at conferring resistance to lymphocyte-mediated rejection should be evaluated in vivo before concluding on the relevance of the approach. Our results also confirm

that high dose STZ can reduce rejection phenomena in beta-cell transplantation, casting doubts on experimental models using high dose STZ-induced diabetes.

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