MINIREVIEW

Autoreactive T-Cell Clones from the Nonobese Diabetic Mouse

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Abstract. **The availability of cloned lines of T cells reactive with islet antigens has provided investigators with new tools to study how T cells contribute to autoimmune disease. T-cell clones isolated from the diabetes-prone nonobese diabetic (NOD) mouse are proving to be particularly valuable for analyzing pathogenesis and hold great promise for determining which T-cell subsets are involved in β-cell destruction versus immunoregulation of the inflammatory process. Diabetogenic T-cell clones have been mostly of the $CD4^+$, Th1 phenotype, but $CD8^+$ T cell clones are also capable of transferring disease. In some cases, T-cell lines and clones ($CD4^+$ and $CD8^+$) have been found to have protective properties. In general T-cell antigen specificities have not been defined, as islet cells or lysates were used as the selecting antigen. However, there is an increasing number of reports of T cells specific for defined islet proteins, such as insulin and GAD, and some of these lines can induce disease. The variety of T-cell clones that have been produced indicate that there may a variety of conditions that lead to or protect against β-cell destruction and provide further evidence that autoantigens are generated during development of disease.** [P.S.E.B.M. 1997, Vol 214]

Insulin-dependent diabetes mellitus (IDDM), also known as type 1 or juvenile onset diabetes, results from the autoimmune destruction of the insulin-secreting β cells in the pancreatic islets (1, 2). Although the disease can be managed through insulin replacement therapy, a significant number of diabetics develop long-term complications, including retinopathy, neuropathy, and nephropathy (3). Disease complications, together with the fact that at the time most diabetics are diagnosed the islet β cells have been largely destroyed (80%–90%), provide a compelling case for intensive research efforts into mechanisms of pathogenesis and regulation of this autoimmune disease.

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Fortunately for investigators who are pursuing answers to these questions, there exists one of the best animal models of autoimmunity, the nonobese diabetic, or NOD, mouse. The NOD mouse was first developed and characterized in Japan (4) and, since the early 1980s, has become widely available and is now the most commonly used animal in studies of IDDM. One reason for its value is that, unlike many autoimmune animal models, disease in the NOD mouse develops spontaneously and does not have to be induced. Another reason is that progression of diabetes in the NOD closely resembles the disease in humans. In both mouse and humans, there is a genetic predisposition to developing IDDM with multiple genes being involved, the most important of which are the Class II genes of the major histocompatibility complex (MHC) (5, 6). The autoimmune nature of this disease is clearly indicated in NOD mice, as it is in human patients, by the presence of antibodies and T cells that are reactive with islet cell autoantigens. There is no indication of a direct role for autoantibodies in the patho-

genesis of diabetes, but the investigation of T-cell involvement has become one of the most active areas of research into autoimmune disease.

Early studies in the NOD mouse indicated that T cells were critical for development of diabetes and included a variety of treatments that prevented disease, including neonatal thymectomy, antibodies to T cells, and immunosuppressive drugs that affect T-cell function. More compelling evidence came from adoptive transfer experiments showing that diabetes could be transferred by T cells from diabetic animals, in both BB rats (7) and NOD mice (8). T-cell depletion studies with antibodies to the T-cell molecules CD4 and CD8 indicated the importance of CD4 T cells in β-cell destruction (9, 10) and that both types of T cells were required for successful transfer of disease by diabetic splenocytes (11, 12). These studies and others have recently been summarized (2, 13, 14) and have firmly established the importance of T cells in the pathogenesis of autoimmune diabetes. Our purpose in this review is to focus on how the isolation of T-cell clones from the NOD mouse has extended the investigation of autoreactive T cells in IDDM and the islet antigens to which they are directed.

Islet-Reactive and Pathogenic T-Cell Clones

There has been a great effort on the part of many investigators to isolate T-cell clones reactive to islet antigens and capable of inducing the disease process. The first report of an islet-specific and diabetogenic T-cell clone was submitted by us and described a clone, BDC-2.5, that could cause islet, but not pituitary, graft destruction (15). Subsequently, we described a panel of $CD4^+$ T-cell clones, all derived from spleen and lymph node cells of newly diabetic NOD mice, that would proliferate and make interleukin-2 (IL-2) in response to islet cells as antigen and NOD splenocytes as antigen-presenting cells (16); properties of these clones are summarized in Table I. T-cell receptor variable gene usage was shown to be heterogeneous (17), although it has turned out that three of seven diabetogenic clones are $V\beta4^+$. Experiments *in vivo* with two of the clones showed that after two or three injections of cells, very young (2- to 3-week-old) unirradiated NOD recipients rapidly developed hyperglycemia and/or extensive insulitis (18). Unlike the more conventional disease transfer studies with diabetic splenocytes into irradiated recipients, our findings with cloned lines of $CD4^+$ islet-reactive T cells suggested that under certain conditions there were no further requirements for disease induction.

Table I. $CD4^+$ Islet-Specific and Diabetogenic T-Cell Clones[a]

Clone	TcR	Islet cell antigen	Diabetogenic
BDC-2.5	Vβ4 Vα1	Mouse	+
BDC-4.12	Vβ19 Vα[4.12]	Mouse	+
BDC-5.2	Vβ6 Vα12	Mouse, rat	+
BDC-6.3	Vβ4 Vα3.1	Mouse	+
BDC-6.9	Vβ4 Vα13.1	NOD, SWR	+
BDC-9.25	Vβ6 Vα(n.d.)	NOD, SWR	+
BDC-10.1	Vβ15 Vα13	Mouse	+

Note. The material in this table is from Haskins *et al.* (15–22, unpublished observations).

In subsequent studies to characterize the *in vivo* activity of these clones, we have demonstrated that all our $CD4^+$ islet-specific T-cell clones tested to date can induce diabetes in mice that are under 3 weeks of age at the time of the first injection of cells. The age-dependent development of diabetes indicated by hyperglycemia is also reflected in histological analysis of the insulitis in experimental animals, with cellular infiltration and β-cell degranulation being much more pronounced in the youngest mice (8–10 days of age) than in slightly older animals (15–18 days of age); no disease could be induced with the T-cell clones in mice more than 18 days of age (19). A similar pattern of age dependence with T-cell clone transfer of disease was observed in several strain combinations of NOD F1 mice, experiments which also documented that these clones were diabetogenic in non–diabetes-prone mice and that expression of the MHC Class II molecule I-E, shown to be protective in development of spontaneous disease, had no effect (20). An extended analysis of cytokine production and disease transfer studies into NOD-*scid* animals have firmly established the Th1-like characteristics of these T-cell clones but have also shown that not all the clones have the same diabetogenic potential *in vivo*. For example, one clone, BDC-6.9, can rapidly and reproducibly initiate diabetes in NOD-*scid* mice, clearly providing a demonstration that a $CD4^+$ T-cell clone can cause diabetes in the absence of any other lymphocytes; however, a second clone, BDC-2.5, only transferred disease in the presence of CD8 T cells isolated from the spleen of a diabetic NOD mouse (21).

Reports from other laboratories (summarized in Table II) indicate that there is considerable diversity in the types and activities of T-cell lines and clones produced from NOD mice. Unlike our clones, which were derived from spleen and lymph node T cells and are all $CD4^+$, almost all of these T-cell lines were isolated from the islets of NOD mice and include $CD8^+$ as well as $CD4^+$ T cells. For example, Reich *et al.* described two cloned T-cell lines derived from NOD islets, one that was $CD4^+$ and the other, $CD8^+$, that responded to NOD islet cells and that could in combination (but not singly) induce extensive insulitis in 7- to 8-week old, sublethally irradiated NOD or (NOD × BALB/c)F1 recipients (22). Another early report of CD8 T-cell lines from NOD islets by Nagata *et al.* (23) described cytotoxic T cells that could specifically kill islet cells. Huber *et al.* reported that islets cultured in IL-2–containing medium produced only $CD8^+$ T-cell lines that showed different patterns of cytotoxic activity on a variety of tumor targets, but none was specific for islet cells; this finding was interpreted as evidence that the majority of IL-2 receptor positive CD8 T cells in islet lesions are not islet specific (24). In a report on the isolation of both $CD4^+$ and $CD8^+$ T-cell clones from NOD islets, Nagata and Yoon found that $CD8^+$ T cells

cytotoxic for islet cells could only be obtained from mice with advanced disease and speculated that $CD4^+$ T cells may be involved primarily in initiation of disease whereas $CD8^+$ T cells are late-stage effectors (25). A later study by this group indicated that the $CD8^+$ CTL lines could induce disease in sublethally irradiated NOD recipients, but only in the presence of $CD4^+$ T cells (26). Maugendre *et al.* (27) used an interesting approach to isolate $CD4^+$ and $CD8^+$ T-cell lines from islet infiltrates in which culture wells were pre-coated with antibodies to TCR Vβ6 or Vβ8; lines with both phenotypes were found to be cytotoxic with CD3-coupled P815 targets or YAC cells, but islet cell specificity was not determined. One $V\beta6^+/CD4^+$ line could induce insulitis in neonatal recipients, but only when injected together with $CD8^+$ T cells. Shimizu *et al.* isolated $CD4^+$ and $CD8^+$ T cell clones from NOD islets that could proliferate and make γ-interferon (IFN-γ) in response to islet cells and NOD APC; two of their $CD4^+$ T-cell clones were diabetogenic, particularly in the presence of splenic $CD8^+$ T cells from diabetic mice (28).

In other studies, $CD4^+$ T-cell clones were predominant. Nakano *et al.* produced $CD4^+$ T-cell clones from islet infiltrates of NOD mice which responded to islet cells from various strains of mice in the context of NOD antigen-presenting cells, showing diverse usage of T cell receptor V and J segments, and transferred insulitis into $I\text{-}E^+$ transgenic NOD mice (29). A somewhat different approach was taken by Prud'homme *et al.* (30), who briefly cultured islet-infiltrating T cells which were then fused with the thymoma BW5147 to yield T-cell hybridomas. Of 94 hybrids, only those that were $CD3^+CD4^+$ made IL-2 in response to islets; among these cell lines, there was a high frequency of islet-reactive hybrids and 12 appeared to be islet-cell specific. Like the work of Huber *et al.* (24), this study suggested that there are many T lymphocytes without specificity for islet antigens in pancreatic lesions. In a report by Pankewycz *et al.* (31), T-cell lines were propagated from pancreatic islets of prediabetic (2-month-old) NOD mice and yielded a $CD4^+$ clone that responded to islet cells and could induce disease in female NOD mice. Another novel approach to deriving islet-specific T cell lines was used by Wegmann *et al.*, who used syngeneic islet grafts in NOD recipients to recruit autoreactive T cells (32). Several lines with islet specificity were obtained and clones from one line, N1, were shown to be diabetogenic in NOD-*scid* recipients. Gelber *et al.* described T-cell lines and clones that were derived from spleen and pancreatic lymph nodes of 30- to 40-day-old NOD females (33). These T cells were reactive with various fractions produced from extracts of a β-cell insulinoma and some of the cloned lines were capable of inducing insulitis in young NOD recipients.

The diabetogenicity of these various $CD4^+$ and $CD8^+$ T-cell lines and clones is apparently quite variable from group to group. In some cases, disease was manifested only in the form of infiltrates, but not as hyperglycemia which indicates overt diabetes (29, 41). In several instances, including our own, $CD4^+$ T-cell lines or clones alone induced disease (18, 28, 29, 32), but in other studies only a combination of $CD4^+$ and $CD8^+$ clones resulted in pathogenesis (22, 25, 27). In the case of islet-reactive $CD8^+$ T cells, investigators have generally not observed disease transfer with $CD8^+$ clones alone, but only when co-transferred with $CD4^+$ lines or spleen cells. However, Wong *et al.* have recently reported on $CD8^+$ T-cell clones isolated from 7-week-old female NOD mice and cultured on islets expressing the B7-1 costimulatory molecule (34). These $CD8^+$ T-cell lines and clones were $H\text{-}2K^d$–restricted and were found to proliferate and be cytotoxic to NOD islets *in vitro*. In adoptive transfer experiments to NOD- or CB17-*scid* recipients, diabetes developed rapidly and in the absence of $CD4^+$ T cells.

Islet-Derived Immunosuppressive T-Cell Clones

In addition to reports of T-cell lines and clones with demonstrated specificity and pathogenicity for islet cells, there have been descriptions of T-cell lines and clones with mostly undefined reactivities, the most interesting of which appear to have protective properties. Several of these showed responses in the presence of NOD spleen cells and no other added antigen; such cell lines have been termed "autoreactive" by some authors, presumably for reactivity with NOD MHC molecules. For instance, Reich *et al.* described a T-cell line isolated from islets that responded to spleen cells or islets from NOD mice, but not other mouse strains, which delayed onset of spontaneous diabetes in NOD recipients (35). In addition to a diabetogenic $CD4^+$ T-cell line, Pankewycz and co-workers isolated a second $CD8^+$ clone, IS-2.15, from pancreatic infiltrates which was not reactive with islet cells but which protected animals from cyclophosphamide-induced diabetes (31). Further work indicated that this protective $CD8^+$ T-cell clone could synthesize mRNA for the immunosuppressive cytokines, IL-10, and growth factor–β transforming (TGFβ) (36). Another protective T-cell clone, C2, of the $CD4^+$ phenotype and reactive with NOD spleen cells, was produced from islet lesions by Chosich and Harrison (37). The C2 clone was found to secrete IFN-γ, tumor necrosis factor (TNF), and IL-6, but not IL-2, IL-4, or IL-10; its proliferation could be blocked by antibody reacting to $I\text{-}A^{g7}$ and it suppressed development of diabetes but not insulitis in cyclophosphamide-treated animals.

Two very interesting observations of $CD4^+$ T-cell lines or clones that were derived from splenic T cells of NOD mice and that showed protective characteristics were just recently described. In the first report of Th2 T-cell lines being isolated from NOD mice, Healey *et al.* described production of $CD4^+$ T-cell lines from splenocytes of diabetic mice, using as antigen a crude membrane fraction from a rat insulinoma, that included T cells with both Th1 (IFN-γ–secreting) and Th2 (IL-4–secreting) characteristics (38). The IFN-γ–secreting lines induced insulitis and diabetes in neonatal NOD recipients, whereas transfer of the IL-4–

secreting lines resulted in nondestructive peri-ductal infiltrates, but no diabetes, within a 30-day period. In contrast to the findings by Healey and co-workers, Akhtar *et al.* (39) reported on the isolation of Th1 $CD4^+$ T cell clones from unprimed 4-week-old NOD mice that could suppress spontaneous development of IDDM or inhibit adoptive transfer of disease. Of the various clones described by Akhtar and colleagues, none were diabetogenic and some could proliferate with NOD spleen cells; however, those clones that were protective were found to be islet-reactive. Most of the clones were Th1 in that they secreted IL-2, IFN-γ, and TNF-α. It is interesting that one Th2-like clone which secreted IL-4, but not Th1 cytokines, showed no protective capacity. Further *in vivo* studies with partially purified culture supernatant fractions suggested that protection against disease by these T-cell clones was mediated by an unidentified secreted factor.

Antigen Specificity of T Cell Clones from NOD Mice

Another way in which T-cell lines and clones produced from the NOD mouse can be categorized is by their antigen specificity: in most cases, antigens are undefined, but in a few instances clones have been propagated with defined islet molecules. A majority of T-cell lines were obtained through culture of T cells, from spleen and lymph nodes, or from islet infiltrates, with islet cells or extracts. Among the variables that may influence antigen specificity are the age of donor mice, the sites from which T cells are obtained, and the nature and source of the antigen preparation.

In general, those groups that have produced T-cell lines and clones reactive with islet cells have not identified the activating antigens. This is not surprising, given the magnitude and difficulty of isolating and identifying islet cell proteins. Extensive characterization of β-cell granules and several granule proteins has been carried out by Hutton and co-workers (40), but there is little information available as to β-cell proteins that are also autoantigens for T cells. With the exception of insulin which is secreted and which, with C-peptide, comprises about 80% of the β cell's protein (40), few other β-cell molecules, particularly those residing in the β granule membrane, have been well characterized. Isolation of mouse islets is very labor intensive and expensive, and a typical yield from an NOD mouse is on the order of 10^5 islet cells; thus, biochemical purification of mouse islet proteins is not feasible without a tumor cell source. Tumor cell lines are known to undergo a variety of changes when kept in culture, and in our case tumor cell lines were without, or quickly lost, antigenicity. Therefore, in order to obtain sufficient islet antigen, we have found it necessary to breed a mouse strain that produces β-cell tumors. Even then a typical preparation from 12–15 mice yields $1–2 \times 10^8$ β cells or 3–5 mg of total membrane proteins, an abundance in terms of β cells, but still meager in terms of a source of protein for biochemical analysis. Thus, it is no wonder that, for the most part, investigators who have propagated islet-reactive T-cell lines do not know the identity of the antigens. Some progress, however, has been made.

In our laboratory, T-cell lines were obtained from spleen and lymph nodes of unprimed, newly diabetic mice and were selected for reactivity with whole NOD islet cells; they were subsequently cloned by limiting dilution under the same culture conditions. We have characterized the antigen specificity of our T-cell clones in stages, first by looking at responses to whole islet cells from different mouse strains, secondly by looking at subcellular fractions obtained from β tumor cells, and finally by testing fractions obtained from sequential biochemical purification procedures. Most of our diabetogenic T-cell clones react to whole islet cells from every mouse strain we have tested (NOD, NOR, NON, BALB/c, C57BL/6, C57L, CBA, SJL, SWR). However, as indicated in Table I, one clone, BDC-6.9 shows strain specificity, responding only to islet cells obtained from NOD mice, the closely related NOR and NON strains, and the SWR mouse. Another clone in the table reacts to rat islets, but none of our clones has shown reactivity to human islet preparations. The antigen specificity of these clones seems to be restricted to islets as other tissue preparations (including spleen, thyroid, and pituitary) do not activate them. The antigen response is MHC-restricted to the $I\text{-}A^{g7}$ haplotype of the NOD, requiring processing by splenic or peritoneal APC. Finally, none of our clones has shown reactivity with a variety of known islet antigens tested, including insulin, glutamic acid decarboxylase (GAD), carboxypeptidase H, heat shock protein, and peripherin (41). Since we found that the antigenicity of β-cell tumor lines was rapidly lost with prolonged *in vitro* culture, we used a β-cell tumor produced in the NOD/Lt-Tg(RIP-Tag)1Lt transgenic mouse (42) as the source of β cells for subcellular fractionation. We were able to separate out a fraction highly enriched β-cell granules that was very antigenic for every islet-reactive T cell clone tested from our panel, and we subsequently localized that activity to the β granule membrane (41).

Over the last 2 years, we have attempted to purify biochemically the antigenic activity from β-cell membranes. This work is described in detail elsewhere (Bergman and Haskins, manuscript submitted), but in brief, we have found that lysates of β granules or whole β-cell membranes can be separated by anion exchange chromatography, with an antigenic fraction eluting off between 0.1 and 0.2 *M* of a NaCl gradient. Interestingly, the antigen activity peaks are superimposable for the two clones, BDC-2.5 and BDC-6.9, despite their differential responses to whole islet cell antigen. When subjected to size exclusion chromatography (HPLC-SEC), the antigenic peak from the anion exchange column, which we are calling β granule–associated protein, or βGAP, was found to fall within a molecular weight range of 50–80 kDa. SDS-PAGE analysis of activity peaks from the SEC column revealed 8–10 bands with silverstain, indicating that a substantial purification of the antigen activity had

been achieved. Further purification steps will be needed before a sample can be obtained for sequencing.

By exploiting the difference in antigen reactivity to whole islet cells observed with two of our diabetogenic T-cell clones, we have also been able to take a genetic mapping approach to identifying the antigen for one of the T-cell clones, BDC-6.9. As mentioned above, this clone reacts only to islet cells from NOD, its related strains, and SWR mice. The clone BDC-2.5 reacts to islet cells from all the inbred mouse strains we have tested. We therefore produced an (NOD × BALB/c) × BALB/c backcross and looked for correlation between chromosomal loci mapped by microsatellite markers and the presence or absence of antigenicity in islets from each backcross mouse. The result was that a locus in the telomeric region of mouse chromosome 6 was found to have a 100% correlation with the presence of the islet antigen in the backcross (43).

Despite the continued efforts by several laboratories, there is still very little information available with regard to the nature of islet antigens for autoreactive T cells. Comparatively greater progress has been made in the investigation of T-cell reactivity with known β-cell proteins such as insulin, heat-shock proteins, and GAD, antigens that were first defined by the presence of autoantibodies in mice and humans. A number of investigators have produced T-cell lines and clones, from primed and unprimed NOD mice, that react to these antigens. T-cell reactivity to heat-shock protein (HSP) was reported by Elias *et al.* (44), and HSP-65–reactive T-cell lines were found to induce disease in NOD mice. Further, vaccination with an HSP-65 peptide was found to protect against development of diabetes in NOD mice (45). More recent studies by Elias and Cohen have indicated that successful peptide therapy in the NOD mouse was associated with downregulation of T-cell immunity to the peptide (p277) and that spleen cells from treated mice were not only not diabetogenic, but could suppress diabetogenicity of spleen cells from untreated mice (46). Work from two laboratories indicated that T-cell reactivity to GAD65, or several GAD65 peptides, could be detected in splenocytes from unprimed NOD mice and that an antigen-specific tolerance could be induced with GAD65 (47, 48). Elliott *et al.* (49) immunized 4-week-old NOD mice with the 67-kDa GAD isoform (GAD67) and found that this form of GAD also prevented or delayed onset of disease. Two T-cell clones were isolated that proliferated in response to NOD APC and GAD, but the diabetogenic potential of these clones was not determined. Insulin-reactive clones obtained from islet infiltrates of unimmunized 7- and 12-week-old NOD mice were described by Wegmann and co-workers (50, 51). An immunodominant peptide epitope consisting of residues 9–23 of the insulin B-chain was identified for these $CD4^+$ T-cell clones, some of which were able to induce diabetes in young NOD or NOD-*scid* recipients (51). A. Cooke and co-workers have isolated T-cell clones from NOD mice with a variety of known antigen specificities, including $CD4^+$ Th1-like clones reactive with insulin or carboxypeptidase H, and $CD8^+$ CTL clones specific for GAD65 and GAD67 (Cooke A, personal communication). Thus, it is clear that T-cell reactivities to a variety of known islet cell proteins arise during development of disease, and some of them have been detected in or isolated from mice as young as 4 weeks. The important question that remains to be answered is whether there is any one T-cell autoantigen important in the initiation of disease.

Concluding Remarks

The availability of cloned lines of T cells from the NOD mouse provides investigators with new opportunities for analyzing the role of T cells in autoimmune disease. We are now able to use defined populations of T cells *in vitro* and *in vivo* to approach questions regarding which T-cell subsets are involved in pathogenesis and regulation. For example, with T-cell clones it has been possible to demonstrate further the importance of $CD4^+$ T cells as prime players in the initiation of disease. Compared with $CD4^+$ T cell clones, relatively fewer attempts at isolating $CD8^+$ T cell clones have been successful, and from most reports describing adoptive transfer experiments with CD8 T-cell clones, it could be concluded that in general CD8 T cells must be accompanied by CD4 T cells for disease to occur. An exception to this rule has recently been provided by Wong *et al.* in their description of diabetogenic CD8 T-cell clones isolated from 7-week-old NOD mice (34). It may be that investigators are getting better at isolating $CD8^+$ T-cell clones and more reports of diabetogenic clones will be forthcoming. Another question that might be posed is whether there are different immunological mechanisms governing different pathogenic clones. Direct cytotoxicity against islet targets has been demonstrated for some $CD8^+$ T-cell clones, whereas $CD4^+$ T cells are generally thought to operate by an indirect mechanism in which cytokines are produced and other cells are recruited. In some cases, however, it may be that $CD4^+$ T cells can directly kill cell targets and that $CD8^+$ T cells are effective only in the presence of $CD4^+$ T cells.

One striking aspect of the reports on NOD T-cell clones is the variety in the methods used to isolate them, particularly with regard to the age of mice used and different culture conditions. Most investigators have used islet infiltrates as a source of T cells, reasoning that the lesion would be the most logical site for islet-reactive T cells. A few laboratories, including ours, have isolated T cells from the spleen and lymph nodes of newly diabetic mice. We have observed that a relatively small percentage of the T cells in the lesion are upregulated for IL-2 receptor, a sign of T-cell activation (Peterson JD *et al.*, manuscript in preparation). This finding, along with the observations of others (24, 30), would suggest that a majority of the T cells in the islet lesion are not islet-specific. We have hypothesized that in the late stages of disease when islet destruction is very advanced, as well as at any other time during the course of disease, it is reasonable to assume that T cells with "memory" for islet antigens may be found in the recircu-

Table II. T-Cell Clones from the NOD Mouse

Origin of T cells	CD4/CD8	Antigen specificity	*In vivo* activity	Investigators
Spleen and lymph nodes of diabetic females	CD4	Islet cells, β granule membrane	Insulitis & diabetes	Haskins *et al.* 1988 (15) Haskins *et al.* 1989 (16) Bergman & Haskins 1994 (22)
Islet infiltrates of diabetic females	CD4 & CD8	Islet cells	Insulitis	Reich *et al.* 1989 (23)
Islet infiltrates of diabetic females	CD4	Unknown	Protection	Reich *et al.* 1989 (24)
Islet infiltrates of 20-week-old nondiabetic females	CD8	Islet cells	n.d.[a]	Nagata *et al.* 1989 (25)
Three-month-old female	CD4	HSP-65	Insulitis	Elias *et al.* 1990 (26)
Islet infiltrates of 8-week-old nondiabetic females	CD4	Islet cells	Insulitis & diabetes	Pankewycz *et al.* 1991 (27)
Islet infiltrates of 8-week-old nondiabetic females	CD8	Unknown	Protection	Pankewycz *et al.* 1991 (27)
Islet infiltrates of 7- to 11-week-old nondiabetic mice	CD4	Islet cells	Insulitis	Nakano *et al.* 1991 (28)
Islet infiltrates of <10- and >10-week-old nondiabetic females	CD4 & CD8	Islet cells	Diabetes (CD8 with co-transfer of CD4 T cells)	Nagata & Yoon 1992 (29) Nagata *et al.* 1994 (30)
Infiltrated islets of an irradiated male recipient	CD4 & CD8	Islet cells	Diabetes (CD4 clones only)	Shimizu *et al.* 1993 (31)
Islet infiltrates of 10- to 12-week-old nondiabetic females	CD4	Unknown	n.d.	Maugendre *et al.* 1993 (32)
Islet infiltrates of 4-week-old nondiabetic females	n.d.	Unknown	Protection	Chosich & Harrison 1993 (33)
Infiltrated islet isografts	CD4	Islet cells	Insulitis & diabetes	Wegmann *et al.* 1993 (34)
Islet infiltrates of 4- and 7-week-old nondiabetic females	CD4	Islet cells, insulin	Insulitis & diabetes	Wegmann *et al.* 1994 (35,36)
Spleen and lymph nodes of 30- to 40-day-old nondiabetic females	CD4	Insulinoma extracts	Insulitis	Gelber *et al.* 1994 (37)
Lymph nodes of 6-week-old or diabetic females	CD4	GAD-67	n.d.	Elliott *et al.* 1994 (38)
Lymph nodes of diabetic females	CD4	Islet cells	Diabetes & insulitis	Healey *et al.* 1994 (39)
Spleen cells of 4-week-old nondiabetic females	CD4	Islet cells	Protection	Akhtar *et al.* 1995 (40)
Islet infiltrates of 7-week-old nondiabetic females	CD8	Islet cells	Diabetes	Wong 1996 (37)

[a] n.d., not determined.

lating lymphocyte pool. Whether there will be differences in pathogenicity or antigen specificity of T cell clones, depending on their tissue source, is yet to be determined.

The antigen specificities of diabetogenic T-cell clones will continue to be an intriguing question for at least the near future. A few clones have been described that react to known islet cell proteins, but the great majority have been cultured with islet cells or cell extracts as antigen and their antigen specificities are as yet undefined. Will it turn out that some antigens are more critical to the initiation of disease and that most others arise as a by-product of β-cell destruction? The diverse islet antigen T-cell reactivities being described are a strong indicator of antigens being generated during the course of disease. There is also the suggestion of a different trend in the reports of NOD T-cell clones reactive with membrane-bound antigens of the β granule. In addition to our panel of clones reactive to β granule membrane proteins, the antigenicity of β-cell membrane proteins for T cells was suggested by the work of Shimizu *et al.* in which paraformaldehyde-fixed islet cells provided T-cell stimulus in the presence of NOD APC and conditioned media from islet cells contained no indication of a secreted antigen (28).

Is the diabetogenicity of $CD4^+$ or $CD8^+$ T-cell clones dependent only on antigen specificity or is it also related to the function and phenotype of the T cell? In general, pathogenic T-cell clones have been reported to be Th1-like in terms of cytokine production, but there are also instances of such clones (both $CD4^+$ and $CD8^+$) being apparently protective. T cells of the Th2 phenotype are thought to be immunoregulatory or suppressive and although there are some indications that clone transfers with these types of

cells do not lead to disease, it is also not clear that they protect from disease. One of the more exciting avenues for exploring the role of clonal T-cell populations in autoimmune disease will be through the use of T-cell receptor (TCR) transgenic mice. The TCR of BDC-2.5, one of the $CD4^+$ diabetogenic clones produced by Haskins *et al.* (15), has successfully been expressed in a transgenic mouse (52). Recent experiments with T cells from the BDC-2.5 TCR transgenic mouse, backcrossed on to the NOD background, indicate that acquisition of Th1 and Th2 functional characteristics can be driven *in vitro* by culturing T cells with appropriate cytokines (53). Interestingly, T cells from the transgenic mouse that were Th2-like in cytokine production were not diabetogenic but also did not prevent disease induced by the Th1 transgenic T cells. Do T cells selected *in vitro* for Th2 cytokine production maintain those properties *in vivo* or do other conditions have to be met in order for them to downregulate Th1 T-cell responses? Opportunities to answer such questions have opened up with the availability of cloned T-cell lines and are sure to be extended even further with mice having monoclonal T-cell repertoires.

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