[Frontiers in Bioscience 13, 6155-6169, May 1, 2008]

Genetic manipulation of islet cells in autoimmune diabetes: from bench to bedside

Yi-Ping Chuang[1], Chi-Hong Chu[2], Huey-Kang Sytwu [3]

[1,3]Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan, ROC, [1,3]Department of Microbiology and Immunology, National Defense Medical Center, Taipei, Taiwan, ROC, [2]Department of Surgery, National Defense Medical Center, Taipei, Taiwan, ROC, [3]Department of Medical Research, Tri-Service General Hospital, Taipei, Taiwan, ROC

TABLE OF CONTENTS

1. ABSTRACT

Type 1 diabetes (T1D) develops via spontaneous autoimmune destruction of pancreatic beta cells. The immunoprogression and effectors of T1D have been determined using non-obese diabetic (NOD) mouse mode. Transgenic mice overexpressing a variety of transgenes driven by insulin promoter demonstrate that both apoptosis and necrosis lead to islet cells death; furthermore, various immune cells and cytokine effectors are involved in the immunoprogression of T1D. Efficiently halting immune attack in the islet milieu by an effector-specific manner apparently provides the preventive and therapeutic strategies in T1D. Islet transplantation has been reported as an appropriate treatment to accomplish insulin independence and long-term homeostasis of glucose in T1D. However, it is difficult to protect the islet grafts from subsequent immune attack and prolong their survival. In this review, we highlight the transgenic mice that express transgenes restricted in islet cells to depict the complicated interactions of immune cells in inflammatory islets, to investigate the protective efficacy of some immunomodulatory genes, and to develop genetically-modified islets tolerant to immune attack that might be used in future clinical application.

2. INTRODUCTION

Type 1 diabetes (T1D) results from a chronic autoimmune destruction against the beta cells in the islets of Langerhans of the pancreas. Substantial decrease in the production of endogenous insulin affects the normal physiological and glycemic control leading to a series of chronic and degenerative complications, such as retinopathy, nephropathy, neuropathy, atherosclerosis, and lipid disorders (1, 2). In such insulin-dependent diabetes mellitus (IDDM), daily treatment with exogenous insulin is required to support life. It is usually characterized by a juvenile onset and currently afflicts about five million individuals in the world (3, 4).

T1D is now investigated as a multifactorial autoimmune disease and both genetic factors and environmental stimuli are involved in the pathogenic process of disease (5, 6). It is a polygenic disease and the human leukocyte antigen (HLA) of major histocompatibility complex (MHC) region on chromosome 6p21 is now known to contain the major loci of T1D. Other genes encoding insulin (INS) on 11p15, CTLA4 on 2q33, PTPN22 on 1p13, the interleukin-2 receptor α chain (IL2RA, also known as CD25) on 10p15 and the IFIH1

(also known as MDA5) on 2q24 are also determined (7, 8). More recent studies have established a significant association between chromosome 12q24, 12q13, 16p13 and 18p11 and T1D (9). Susceptibility to T1D is strongly determined by HLA DQ beta alleles that encode serine, alanine, or valine at position 57 on both chromosomes (10). DQ beta alleles positive for aspartic acid at position 57 mediate resistance to T1D. Also, HLA DR beta1-DQ beta1 alleles lacking aspartic acid at position 57 are associated with a higher degree of T1D susceptibility in Japanese patients (11). Peptide elution studies by Ramensee, *et al.* provided evidence indirectly supporting the hypothesis that HLA-DQ and -DR polymorphisms mediate the susceptibility to IDDM by selectively affecting the nature of the peptides presented to T cells by these MHC II molecules (12). Thus far, several autoantigens targeted in T1D are clarified, including insulin, GAD65, GAD67, *et al.* (13).

In 1980, T1D animal model has been established from outbred Jcl:ICR mice that exhibit cataract typical for diabetic patients (14). The resulting non-obese diabetic (NOD) mouse model reveals polydypsia, polyuria, hyperglycemia and insulin deficiency. Diabetic frequency is distinct between different genders in NOD mice. Cumulative incidence in female mice is 80-90% by the age of 30 weeks, but only 20% in male mice (15). Similar to human patients with human insulin-dependent diabetes mellitus (IDDM), NOD mice reveal disease-associated polymorphisms in MHC II I-A beta genes and succumb to a disease process resembling the human patients (16, 17). According to the high similarity, plenty of the knowledge of immunopathogenesis about T1D is derived from the NOD animal model. The progress of the disease seems to be happening through two key breakdowns. From 3-4 weeks of age, inflammatory cells infiltrate into the pancreas starting as the non-invasive peri-islet infiltrate, termed insulitis. However, the infiltration turns into an aggressive state with time, and significant beta cell destruction as well as consequent diabetes is evident around 10-12 weeks of age. The infiltrated inflammatory cells consist of T cells, B cells, macrophages, and dendritic cells (DCs). Accumulating evidence reveals that the development of T1D is the complex multi-cellular interactions of these cells.

Results from adoptive transfer experiments indicate that both CD4 and CD8 T cells are necessary for T1D development (18). Based on the analyses of human pancreatic biopsy, it has been reported that both MHC I and II molecules are aberrantly expressed on beta cells in association with immunoreactive interferon-alpha, however, these aberrant expressions are often observed in the absence of an inflammatory infiltrate. Therefore, it has been suggested that the upregulation of MHC I and II is an early event before the development of insulitis, and it may not be associated with the cytokines produced by mononuclear cells. In parallel, results from transgenic mice expressing syngenic H-2K (MHC I) in B10 background or I-A alpha/I-A beta (MHC II) in BALB/c background restricted in beta cells failed to prove the correlations between diabetic incidence and immune attack, since no autoimmune reaction and lymphocyte infiltration have been observed in the islets (reviewed in 19 and 20). However, due to the lack of equal amount of beta-2-microglobulin co-expression, transgenically overexpressed class I heavy chain failed to be transported to cell surface, making the results inconclusive (21). Thus, these results can only imply that priming or induction of CD4 T cells is not necessarily associated to the abnormal expression of MHC II on beta cells.

Priming of these beta cell-specific autoreactive T cells seems to need pancreatic lymph node (PLN) (22). Results from both BDC2.5 and 8.3-NOD models, transgenic mice carrying beta cell antigen-specific TCR Vα1Vβ4 CD4 T cell clone or Vα17Jα42 CD8 T cell clone respectively, have demonstrated that these autoreactive T cell clones were primed exclusively in PLN (23, 24) and this process did not occur in neonatal stage (24). It has been proposed that this priming process is possibly associated with the shedding kinetics of beta cell antigens in NOD mice. In 2000, Trudeau *et al* observed that priming of 8.3-$CD8^+$ or BDC2.5-$CD4^+$ T cells in NOD mice is preceded by a physiological wave of beta cell apoptosis, which peaks at about 2 weeks of age (25). Therefore, it has been proposed that death of beta cells facilitates the entry of antigens into the cross-presentation pathway (23); however, shedding of beta cell autoantigens to the cross-presentation pathway in autoimmune diabetes is not T cell-dependent (26). All these results strongly suggest that the shedding and presentation of beta cell antigens in PLN of juvenile animals may be the initial step of the first “checkpoint” in diabetic progression that results in insulitis.

After priming, effector T cells as well as other lymphocytes, dendritic cells (DC) and macrophages drain into the pancreas. The peri-islet infiltration of immune cells turns to be aggressive until 10-12 weeks that evokes significant immune cell attraction. There might be complicated interactions among cells and effectors in islet milieu over this period. Transgenic mouse models overexpressing a variety of genes in beta cells provide useful tools to uncover these complicated interactions and explain this aggressive switch. Via a variety of transgenes expressed locally in beta cells, autoimmune process-related cytokines, adhesion molecules or other mediators are thus far investigated to have definitive roles in the immunopathogenesis of T1D. Furthermore, counter-regulation factors/antagonists can also be evaluated in these transgenic mice for their potential as therapeutic targets in the future.

3. TRANSGENIC MOUSE MODEL

Transgenic mice are created by injecting purified DNA into male pronuclei of one-celled mouse embryos and followed by re-implanting these embryos into the oviduct of pseudopregnant female mice and allowed to continuous development (Figure 1) (27). The developed embryos may incorporate the injected DNA into chromosome. The integration must take place before pronuclei fusion. Once it integrates in or after the stage of embryo cleavage, the resulting transgenic mice will be mosaic (28). The

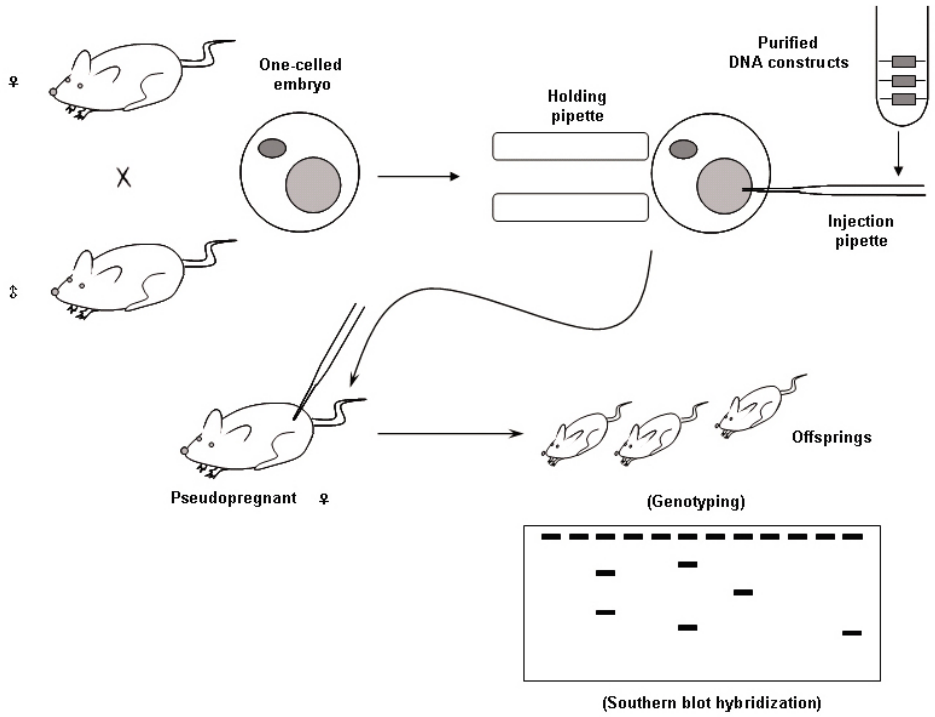

Figure 1. Generation of transgenic NOD mice. Superovulated female NOD mouse is mating with male one and the one-celled embryos at the stage that nuclei from egg and sperm have not fused are then collected. Purified DNA constructs are delivered into male pronuclei in the one-celled embryos via microinjection followed by re-implanting these embryos into the oviducts of pseudopregnant female mice, allowing them to continuous development. The offsprings are then genotyped by Southern blot hybridization.

integration is usually random and ranging from one to several hundred copies. DNA from those mice will then be extracted to confirm the existence and the copies of transgene by Southern blot analysis. In theory, the transgene should be stably expressed in the offsprings, though rearrangements of the tandem array have been reported (29, 30).

Insulin promoter is a well-defined and pancreas-specific promoter regulated by complex signaling-controlled transcription factors, such as PDX-1, MafA, E47/b2, associated with cAMP regulation (31). Although low level of insulin has been detected in some extra-pancreatic tissues such as brain and thymus, the insulin promoter is the most popular one driving transgene restricted in beta cells. In some cases, insulin promoter-driving transgenes can also be detected in kidneys. Most of the literature on the insulin promoter pertains to rat insulin promoter (RIP) and human insulin promoter (HIP) that efficiently drive transgene expression in the pancreas. Recently, sequences of insulin promoters from various organisms were compared and emphasized the dissimilarity between rodent and human insulin promoters (32). It might give some take-home messages about the consequences of the thus far generated transgenic animal systems, not merely expression level, transgene distribution but also kinetics of regulation.

The transgenic mouse models that ectopically expressed various transgenes in islet cells described in this review (summarized in Table 1) help to dissect the immunopathogenic and immunomodulatory roles of effectors in T1D. We here not only depict the correlations of these transgenes with immunopathogenesis of T1D but also investigate the therapeutic potential of counter-regulation factors/antagonists-based genetically-modified islet grafts in the future.

4. IMMUNOPATHOGENESIS OF T1D

4.1. Pro-inflammatory mediators and antagonists

It has been well characterized that there are elevated pro-inflammatory cytokines such as IL-1beta, IFN-gamma, TNF-alpha in inflamed pancreas (33-35) which are mainly produced by macrophages and CD4 T cells (36-38). The existence of pro-inflammatory cytokines mimics the situation of viral infection, leading to MHC I (39, 40), II (41, 42) and Fas (43, 44) expressions on islet cells and promoting beta cells to generate reactive oxygen intermediates (ROIs) including NO, OH, H_2O_2, and O_2^- (45-48). ROIs, either administered exogenously or induced in beta cells by cytokines, have been demonstrated to cause beta cell destruction *in vitro*, possibly via apoptosis (49-53).

The susceptibility of beta cells to these inflammatory situations and the targeting therapeutic candidates have been determined through various

Table 1. Transgenic mice express transgenes under insulin promoter control

Transgenic mice	Insulitis	Diabetic process and incidence	Reference
B6.RIP-TNFalpha	Yes	None	55, 56
NOD.RIP-TNFalpha (neonatal)	Yes (massive)	Accelerated	58
NOD.RIP-TNFalpha/beta2m$^{-/-}$	None	Prevented	59
NOD.RIP-TNFalpha/CIITA$^{-/-}$	Yes	Yes (similar with its parental strain)	59
NOD.RIP-TNFalpha/CD154$^{-/-}$	Yes	Yes (similar with its parental strain)	59
NOD.RIP-TNFalpha	Yes (massive)	Ameliorated	62
NOD.HIP-TRX	Yes	Delayed and ameliorated	71
NOD.HIP-HO-1	Yes	Delayed and ameliorated	Unpublished data
BALB/c.HIP-IFN-gama	Accumulation of inflammatory cells within the parenchyma of pancreas rather than insulitis	Yes	20, 65
NOD.HIP-IL-4	None (rarely peri-insulitis)	Prevented	87
BDC2.5-NOD.HIP-IL-4	Yes	Accelerated	89
NOD.HIP-TGFbeta1	Yes (peri-insulitis)	Prevented	95
NOD.RIP-B7.1	Yes	Accelerated	108
NOD.RIP-B7.1/beta2m$^{-/-}$	None	Ameliorated	108
NOD.RIP-B7.1/CD4$^{-/-}$	Yes	Ameliorated	108
NOD.RIP-B7.1/mMT$^{-/-}$	Yes	Yes (similar with its parental strain)	108
B6.RIP-B7.1	None	None	109
B6.RIP-B7.1/H-2$^{g7/g7}$	Yes	Yes	109
BALB/c.RIP-B7.1	None	None	109
NOD.RIP-E3	Yes	Delayed and ameliorated	106
B10.RIP-H-2K^{b}	None	Yes	155
BALB/c.HIP-I-A	None	Yes	20
(B6xSJL)F1.RIP-I-E	None	Yes	156
NOD.HIP-PD-L1	Yes	Delayed and ameliorated	Unpublished data
NOD.HIP-anti-CTLA4 scFv	Yes	Delayed and ameliorated	Unpublished data
NOD.RIP-FasL	Yes	Accelerated	52
NOD.HIP-FasL	Yes	Accelerated	130
NOD.HIP-DcR3	None (almost free)	Prevented	133
NOD.HIP-anti-4-1 BB scFv	Yes (augmented)	Accelerated	142
NOD8.3.RIP-SOCS-1	Yes	Prevented	80

Abbreviation: NOD, non-obese diabetic mice; B6, C57BL/6; RIP, rat insulin promoter; HIP, human insulin promoter; TRX, thioredoxin; HO-1, heme oxygenase 1; E3, adenovirus early region 3; DcR3, decoy receptor 3; PD-L1, programmed death 1 ligand 1; SOCS-1, suppressor of cytokine signalling-1; CTLA-4, cytotoxic T lymphocyte-associated

transgenic mouse models. Respective antagonists have also been provided as the potential tools against T1D.

4.1.1. TNF-alpha

The kinetic expression of TNF-alpha in islets has already been shown to be accompanied with the progression of autoimmune diabetes (54). To explore the role of TNF-alpha in autoimmune diabetes, B6 mice bearing TNF-alpha transgene restricted in beta cells were generated showing severe lymphocytic infiltration, but no diabetes (55). The inflammatory cells infiltrated in islets composed of CD4 and CD8 T as well as B lymphocytes; however, this insulitis does not progress with age (56). These results suggest that inflammation induced by TNF-alpha is not sufficient to progress islet destruction in non-NOD mice.

TNF-alpha has multiple functions in the development of the immune system. In addition to serve as the pro-inflammatory cytokine, it also modulates the adaptive immune responses. Early administration (from birth to days 21-24) of nontoxic doses of TNF-alpha via i.p. injection results in an increased incidence and early onset of diabetes in female NOD mice (57). Coincidence with early TNF-alpha administration outcome, neonatal RIP-TNF-alpha transgenic mice reveal an accelerated onset of diabetes by provoking influx of antigen-presenting cells to present islet peptides to activate autoreactive T cells (58). Results from this transgenic model also demonstrate that the rapid onset of diabetes in RIP-TNF-alpha mice is CD8 T cell dependent and not associated with CD4 T cells as well as CD40-CD154 signaling (59). Furthermore, neonatal treatment with or blockade of TNF-alpha demonstrates that TNF-alpha would skew DC subsets and affect the DC maturation (60).

In contrast, a reduced incidence and a significant delayed onset of diabetes occurred in NOD mice administered with TNF-alpha at 4 weeks of age or later for a period of 3 weeks (61). Consistent with this, RIP-TNF-alpha transgenic mice generated by Grewal, *et al*, which expressed TNF-alpha locally in islets at 7 weeks of age or later, were protected from IDDM (62).

These results indicate that in addition to serve as a pro-inflammatory mediator, TNF-alpha can positively or negatively regulate peripheral tolerance of T cells against beta cell antigens in NOD mice, possibly through the modulation of DC functions in an age-dependent manner.

4.1.2. IFN-gamma

IFN-gamma is produced mainly by T lymphocytes and natural killer cells in response to viral and bacterial infections. IFN-gamma has been proved to enhance the immunomodulatory function of antigen-presenting cells and cytotoxicity of natural killer cells; it can also recruit lymphocytes to perform their effector functions (63). Furthermore, it has been found that IFN-gamma expression is closely correlated with islet cell destruction (64). HIP-IFN-gamma transgenic BALB/c mice

developed diabetes by the influx of inflammatory cells and progressive destruction of pancreatic islets (20). Moreover, result that lymphocytes from HIP-IFN-gamma transgenic mice are cytotoxic to normal islets *in vitro* suggests that pancreatic expression of IFN-gamma alters immune regulation and results in lymphocyte activation (65). Consistent with this, treatment of NOD mice with anti-IFN-gamma mAb prevented the cyclophosphamide-induced T1D as well as the adoptive transfer-mediated diabetes (66). Because IFN-gamma can synergize with other lymphokines to upregulate the expressions of MHC I, II and Fas molecules (reviewed in 63 and 67), these results demonstrate that IFN-gamma also plays a role in the activation of autoreactive T lymphocytes leading to subsequent cytotoxicity.

4.1.3. Thioredoxin (TRX)

Thioredoxin (TRX) contains conserved vicinal cysteine residues in CXXC motif that are kept in reduction state by the NADPH-dependent seleno-flavoprotein, thioredoxin reductase (68). It facilitates the reduction of other proteins by cystein thiol-disulfide exchange. It has been shown that TRX is induced by various types of stress, such as virus infection, X-ray irradiation, and H_2O_2; it acts as a scavenger of ROIs and repairs proteins oxidized by them (69, 70). To directly assess the implications of oxidative stress in T1D, Hotta *et al.* have generated transgenic NOD mice with TRX overexpression in pancreatic beta cells (71). The incidence of diabetes is dramatically reduced, whereas insulitis development is not prevented. Moreover, induction of diabetes by streptozotocin, a ROI-generating agent, is also abated in these TRX-expressed transgenic NOD mice. These results strongly suggest that oxidative stress is an essential factor involved in the destruction of beta cells in autoimmune T1D. Our current results also demonstrate that NOD islets transduced with lentiviral vectors carrying TRX gene significantly prolong the graft survival in diabetic recipients, further supporting a protective role of TRX in beta cells against immune attack (unpublished data).

4.1.4. Heme oxygenase-1 (HO-1)

The heme oxygenase-1 (HO-1) gene is commonly induced by agents and chemicals that produce an oxidative cellular stress involving the generation of reactive oxygen species (ROS). In this regard, HO-1 induction has been recognized as a general response to oxidative stress. HO-1 catalytically converts its substrate, heme, into three principle reaction products, iron, carbon monoxide (CO) and biliverdin which is subsequently reduced into bilirubin by biliverdin reductase (reviewed in 72). These metabolic side products mediate an efficient anti-oxidant effect and anti-apoptosis via MAPK pathway and activation of NF-kB (reviewed in 73). It has been shown that long-lasting expression of HO-1 by intermittent administration of CoPP decreases blood glucose and pancreatic O_2□, and increases cell survival in NOD mice (74). Meanwhile, single intravenous injection of a rAAV carrying HO-1 gene reduced destructive insulitis and the incidence of overt diabetes (75). It has been shown that reduction of $CD11c^+$ DC population may be part of the reason leading to protection. It might be interesting to transgenically express HO-1 locally in pancreatic islets against immune cell-induced oxidative stress without affecting CD11c+ DC percentage shown in NOD mice with systemic HO-1 expression. Our laboratory has generated such transgenic NOD mice and demonstrated a significant protective role of HO-1 in beta cells; the application of HO-1-overexpressing islets for transplantation is currently under investigation (unpublished data).

4.1.5. Suppressor of cytokine signaling-1 (SOCS-1)

Pro-inflammatory cytokines such as IFN-gamma induce Janus tyrosine kinase (JAK)-signal transducer and activator of transcription-1 (STAT1) signaling in beta cells (39, 76). This activation pathway subsequently upregulates Fas, MHC molecules as well as inducible nitric oxide synthase (iNOS) that contribute to the pathogenesis of T1D leading to the destruction of beta cells. Moreover, they also induce chemokine expression, especially CXC ligand 9 and 10, by beta cells that might be involved in the development of insulitis (77, 78). Suppressor of cytokine signaling-1 (SOCS-1) has been shown to inhibit JAK kinase, prevent STAT1 signaling (79) and subsequently attenuate IFN-gamma transduction (39). SOCS-1 is primarily expressed in beta cells (39). Islets from C57BL/6-129sv mice deficient in SOCS-1 are more sensitive to TNF-alpha- and IFN-gamma-induced cell death (76). TCR transgenic NOD8.3 mice overexpressing SOCS-1 on beta cells are completely free from diabetic development, indicating a protective role of SOCS-1 in beta cells (80). The result also demonstrates that the protection is contributed at least in part by inhibiting TNF-alpha and IFN-gamma-induced Fas expression on beta cells. Additionally, IFN-gamma-induced MHC I upregulation and TNF-alpha- and IFN-gamma-induced IL-15 expression by beta cells are also inhibited by SOCS-1. These results strongly suggest that increase of SOCS-1 expression in beta cells should be a powerful strategy to block cytokine mediated inflammation followed by CD8 T cell-mediated cytotoxicity in T1D.

4.2. Th1/2 counter-regulation

The immune system is dynamic, however, maintained in balance among various types of T lymphocytes. Disturbance of this homeostasis could lead to dominance of a specific effective response and provoke autoimmune disease. The CD4 T lymphocytes include two major types, Th1 and Th2 subsets. Th1 cells release predominantly IFN-gamma, which has been considered being involved in pathogenic autoimmunity, such as experimental autoimmune encephalomyelitis (EAE) and T1D. In contrast to the Th1 cells, Th2 cells producing IL-4, IL-5, IL-6, IL-10 and IL-13 are associated with allergic response and presumably dampening the Th1 effective response. It has been shown by animal studies that administration of cytokines to promote Th1 cell development exacerbates the autoimmune diabetes and that monoclonal antibodies against Th1-derived cytokines block the development of the disease (81). In addition, beta cell-specific T cell clones that skewed to Th1 phenotype could efficiently transfer disease into young NOD recipients (82-84). Th2 cells characterized by the secretion of IL-4, 5, 6, 10 and 13 are mainly involved in humoral immunity and play critical role in the suppression of IDDM.

Administration of IL-4 (85) or IL-10 (86), that promotes Th2 development, protects NOD mice from diabetes. The following transgenic mice, originally designed to explore the modulatory roles of Th1/2 cytokines in immunopathogenesis of autoimmune diabetes, unravel more pleiotropic functions of these cytokines besides Th1/2 balance.

4.2.1. IL-4

In situ expression of IL-4 within the islets could alter the diabetogenic properties of T cells that infiltrate to the pancreas and trigger them to differentiate toward a nondestructive phenotype. It has been demonstrated that expression of IL-4 in beta cells completely protects NOD mice from insulitis and autoimmune diabetes (87). Lymphocytes from these transgenic mice present the tolerant properties that fail to either destroy syngenic islet grafts or to provoke diabetes by adoptive transfer experiments. Transgenic expression of IL-4 in beta cells attenuates the effector function and pathogenicity of autoreactive T cells in a systemic manner (88). The mechanism underlying IL-4 counter-regulation seems in part depend on overwhelming Th1 activity. HIP-IL-4 transgenic NOD mice are also protected diabetes against cyclophosphamide injection, which can upregulate IFN-gamma expression by pancreatic lesion and favor Th1 activity. In addition, it has also been reported that IL-4-mediated counter-regulation is T cell repertoire-dependent. Pancreatic expression of IL-4 activates islet antigen-specific BDC 2.5 T cells and triggers IDDM in HIP-IL-4/BDC2.5 double transgenic mice. It is considered that local expression of IL-4 increases self-antigen presentation by DCs and macrophages resulting in accelerated development of autoimmune diabetes in these double transgenic mice (88, 89). This result also implies that, in addition to effector T cells with enough diversity of their T cell receptor repertoires, the regulation of immune tolerance within pancreas would require some other types of T cells such as nature killer T (NKT) or regulatory T (Treg) cells (reviewed in 90 and 91).

4.2.2. TGF-beta

It has been well characterized that transforming growth factor beta (TGF-beta) plays an important role in anti-inflammation (92). This cytokine inhibits the production of pro-inflammatory and effector cytokines from macrophages and lymphocytes. Additionally, TGF-beta downregulates MHC II expression on macrophages and B cells (93), and may participate in modulating the differentiation of CD4 effector T cells (94). Recently, many efforts are focused on the physiological tolerance mediated by TGF-beta that either induces the differentiation of adaptive regulatory T cells in periphery or negatively regulates the production of Th1 cytokines such as IFN-gamma (reviewed in 91).

Local expression of TGF-beta in the pancreatic islets protects NOD mice from T1D via polarization of GAD-specific response towards an IL-4-producing Th2 phenotype (95). Splenocytes from HIP-TGF-beta transgenic NOD mice reduce the diabetic frequency in transferred NOD.scid recipients. However, splenocytes isolated from transgenic donors treated with anti-IL-4 antibody lose their protective potential when transferred into recipients. The result also demonstrates that TGF-beta expressed in islets expands intra-islet but not splenic FoxP3-expressing $CD4^{+}CD25^{+}$ regulatory T cells (96). Interestingly, T cell receptor repertoires of splenic T cells isolated from TGF-beta transgenic mice are decreased. Although the mechanisms driving the Th2 polarization and diminished T cell receptor repertoires are still unclear, the data from TGF-beta transgenic mice suggest a novel regulatory role of TGF-beta on CD4 T cell differentiation in NOD mice, and imply an unraveling complicated cellular interaction during immunopathogenic process in autoimmune diabetes.

4.3. Contact-dependent effectors and antagonists

As described previously, immune cells infiltrated into pancreas composed of CD4 T cells, CD8 T cells, B cells, DCs, and macrophages. At first, disease is initiated by infiltration of antigen-presenting cells, particularly DCs, into pancreatic islets followed by CD8 T cells that recruited earlier than CD4 T and B cells (16, 97, 98). Previous experiments demonstrated that CD4 and CD8 T lymphocyte are both essential for adoptively transferring diabetes (99, 100). Depletion of either T cell subset fails to transfer diabetes into young irradiated NOD recipients. However, later experimental findings revealed that either splenic CD4 T cells from NOD mice or cloned islet-reactive CD4 T cells can transfer islet inflammation (insulitis) into immunocompromised hosts independent of CD8 T cells (18, 82, 101). This result highlights the "dominant" diabetogenic ability of CD4 T over CD8 T cells. However, NODβ2m^{null} mice deficient in MHC I expression leading to a poor development of CD8 T cells develop neither insulitis nor diabetes (102-105). Moreover, the incidence of diabetes is significantly decreased in transgenic mice locally expressing adenoviral early 3 (E3) region in beta cells that interferes with MHC I-mediated antigen presentation and inhibits cytokine-induced apoptosis (106). Furthermore, perforin-deficient NOD mice reveal reduced incidence and delayed onset of diabetes (107), suggesting that perforin-dependent cytotoxicity is crucial for the beta cell destruction in effector phase and the process is possibly contributed mainly by CD8 T cells. These results highlight the importance of CD8 T cells rather than CD4 T cells for the destruction of beta cells in T1D.

More recently, a report demonstrates that local expression of B7-1 transgene on pancreatic islets in NOD mice converts the beta cells into effective APCs resulting in a rapid and severe autoimmune attack (108). In 2005, Wong, *et al.* generated B6.H-2^{g7}/RIP-B7-1 mice and successfully proved that expression of B7-1 on islets promotes the diabetic progression in its congenic B6.H-2^{g7} mice (109). Splenocytes from B6.H-2^{g7}/RIP-B7-1 could transfer autoimmune disease into NOD.scid/RIP-B7-1 but not NOD.scid mice. It seems that local expression of costimulatory molecule B7-1 is critical for autoreactive CD8 T cells to break tolerance. Although immunohistochemical analyses of NOD pancreatic sections revealed no evidence of B7-1 or B7-2 expression on beta

cells prior to the onset of either spontaneous or cyclophosphamide-induced diabetes, these transgenic mouse models still provide an insight into the pathogenic mechanism mediated by CD8 T cells in autoimmune diabetes (110). However, the fact that a relatively low number of BALB/c/RIP-B7-1 (4/35) and none of B6/RIP-B7-1 (0/40) mice developed autoimmune diabetes for 30 weeks also emphasizes the significance of MHC II and CD4 T lymphocytes in the development of T1D.

Whatever CD4 or CD8 T cells, much current attention has been focused on how to efficiently prevent destructive behavior of autoreactive T lymphocytes that infiltrate into the pancreas. For this purpose, many immune-regulatory molecules have been tried to express on beta cells in order to downregulate activated T lymphocytes. These transgenic models help to dissect the activation phase of antigen-specific T cells as well as to verify the respective tolerance efficacy of each regulatory molecule on T1D.

4.3.1. Programmed death 1 ligand 1 (PD-L1)

Programmed death 1 (PD-1) is inducibly expressed on activated T lymphocytes and provides an inhibitory signal that shapes the immune response. One of its ligands, programmed death 1 ligand 1 (PD-L1), is expressed not only on hematopoietic immune cells but also on non-hematopoietic cells such as vascular endothelial cells and pancreatic islet cells (111). There is cumulative evidence that PD-L1 plays a significant role in peripheral tolerance (112-114). To efficiently suppress the islet-responsive T cells either CD8 or CD4 T cells, local expression of inhibitory signals on beta cells seems fascinating. It has been reported that PD-1-PD-L1 axis not only inhibits the induction of self-reactive T cells but also maintains peripheral tolerance in NOD mice (115). Moreover, PD-L1$^{+/+}$ but not PD-L1$^{-/-}$ islet graft prolongs the physiological function against self-reactive T cells in diabetic mice (116). Our laboratory has currently generated transgenic NOD mice locally expressing PD-L1 on beta cells to enhance the suppressive function against autoimmune T cells. Our results reveal a significant protection of these transgenic mice against autoimmune diabetes (unpublished data). All results described above emphasize the potentials that overexpression of inhibitory molecules on beta cells would enhance an impressive immune tolerance including antigen-specific suppressive effect and maintenance of the immune homeostasis.

4.3.2. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4)

The homologous proteins CD28 and CTLA-4 (CD152) play opposing effects on T cell activation. CD28/B7 interaction serves as a positive costimulatory signal upon TCR engagement with peptide/MHC complex (117). In contrast, CTLA-4/B7 interaction negatively regulates T cell activation by downregulating the ongoing T cell response such as cell cycle progression, IL-2 production, and T cell proliferation upon activation (118, 119). Now it has been well characterized that CTLA-4 plays a critical regulatory role both in the induction and maintenance of immune tolerance (120, 121). Administration of anti-CTLA-4 mAb seems to act as an antagonistic function by blocking CTLA-4/B7 interaction and thus enhancing immunity on T cell activation (122-126). It has been reported that transduction of negative signaling via CTLA-4 by using CTLA-4-specific single chain antibody (scFv) requires cross-linking of CTLA-4 molecules in conjunction with T cell receptor engagement either by anti-CD3 or MHC/peptide complex (127). Thus anti-CTLA-4 scFv could successfully act as a CTLA-4 agonist accompanying with TCR signaling. This approach has been applied onto the therapy for autoimmune diabetes by expressing this single chain antibody on B cell (128). It could be a potential approach to express anti-CTLA-4 scFv on pancreatic beta cells combined with MHC/self antigen complex to deliver an inhibitory signal into activated T cells and block their effector function. Our unpublished results about transgenic NOD mice locally expressing this membrane-bound single chain anti-CTLA-4 molecule on beta cells indicate that tissue-specific overexpression of this molecule effectively protects islet from autoimmune T cell attack (unpublished data).

4.3.3. FasL

Peripheral tolerance is maintained through the deletion of autoreactive lymphocytes (induction of apoptosis), functional inactivation (anergy), or suppression of cell activation via regulatory T lymphocytes. Homeostasis of immune system mediated by the induction of apoptosis is maintained in part by members of the tumor necrosis factor (TNF) and TNF receptor (TNFR) family (reviewed in 129). Fas and FasL are both expressed on activated T lymphocytes. Activated T cells might undergo apoptosis when meet another T cells with FasL. Some efforts have been made to overexpress FasL on pancreatic islets and to see whether this strategy can “kill” the infiltrated T cells in pancreatic islets, but unexpectedly those transgenic NOD mice revealed higher susceptibility to the development of diabetes than their control littermates (52, 130). It has been demonstrated that during the natural course of autoimmune diabetes, beta cells would also express Fas by the induction of T cells and inflammatory cytokines leading to subsequent cell death via Fas-FasL interaction (52, 131, 132). These transgenic beta cells then committed to suicide rather than be protected.

4.3.4. Decoy receptor 3 (DcR3)

Considering about the risk caused by the overexpression of FasL in beta cells discussed above, we have tried to adopt a soluble form of death receptor, decoy receptor 3 (DcR3) and to investigate its potential in preventing beta cell damage (133). DcR3 is a soluble death decoy receptor belonging to the TNFR family, and it blocks death signaling mediated by several other TNFR family members, such as Fas and LTbeta receptors. It has been well characterized that soluble DcR3 directly interacts with FasL (134), LIGHT (135), or TNF-like molecule 1A (TL1A) (136), and thus neutralizes their biological effects such as cytotoxicity. Transgenic NOD mice bearing DcR3 specifically expressed on beta cells (HIP-DcR3) were significantly protected against spontaneous, cyclophosphamide-induced, and pathogenic T lymphocyte-transferred diabetes (133). Moreover, transgenic islets expressing high level DcR3 reveal a higher transplantation

success rate and longer survival in diabetic recipients, compared with wild type grafts (133).

It has also been demonstrated that NOD mice transgenic for a dominant-negative Fas in beta cells initiated an early interference in the Fas pathway and prevented from autoimmune diabetes (137). Moreover, blockade of LIGHT signaling by one of its soluble receptors, HVEM-Ig, ameliorated the severity of spontaneous autoimmune diabetes (138). Furthermore, LIGHT blockage significantly inhibited anti-CD3-induced T cell proliferation *in vitro*. Therefore, it is very likely that DcR3 protects beta cells from apoptosis by neutralizing the FasL- or LIGHT-bearing cell-mediated cytotoxicity and thus preserves the integrity and function of islets.

4.3.5. 4-1BB

4-1BB (CD137), another member of TNFR superfamily, is a costimulatory receptor primarily expressed on activated T cells and natural killer cells in human, and also on unstimulated splenic DCs in mice. 4-1BB signaling augments T cell proliferation and cytokine production through both CD28-dependent and -independent mechanisms (139). An anti-4-1 BB mAb has been shown to inhibit T cell-dependent antibody response, presumably by inducing anergy in T helper (Th) cells, either directly or indirectly through induction of regulatory T cells (140). The use of an agonistic mAb in a mouse model of EAE results in a significant decrease in incidence and severity of disease (141). However, our results demonstrate that transgenic NOD mice that express an agonistic single-chain anti-4-1 BB Fv on the surface of beta cells presumably to induce the apoptosis of T cells unexpectedly reveal a faster and severer diabetes and higher mortality rate (142). The detailed mechanism is not clear now, and 4-1 BB-4-1 BBL axis might play more complicated role in T cell proliferation and immune function in T1D mouse model than reached so far.

5. ISLET TRANSPLANTATION

T1D affects millions of individuals worldwide with failure of glycemic control that leads to renal damage, retinopathy, neuropathy, heart disease, and circulatory complications. It has been reported that insulin therapy alone could not prevent these complications. Thus, the methods of efficiently preserving islet function and maintaining physiological glucose control are critically needed in clinical settings. Although Edmonton protocol is now established to successfully restore long-term endogenous insulin production and glycemic stability, insulin-independence could not be sustained overtime after the final transplantation (143). Finally, the issue of life-long immunosuppressive needs must be considered in all recipients following transplantation. It has been estimated that 60-70% of the transplanted beta cell mass may be lost in the early post-transplant period in both immunodeficient and syngenic islet transplantation models (144-146). It has also been suggested that non-immune mediated physiological stress such as hypoxia, ROS or instant blood-mediated inflammatory reaction (IBMIR) induces platelet activation, clot formation and performs the initial wave of islet graft demise (147, 148). Thus, islet grafts must be equipped with the protective abilities against oxidant stress, apoptosis, and subsequently against autoimmune destruction, leading to a better and longer survival.

Transgenic mice bearing protective transgenes expressed in pancreatic islets are powerful tools for evaluating their protective effects and mechanisms in T1D. Moreover, counter-regulation mediated by these transgenes could be monitored systemically; on the other hand, these transgenic islets can easily be isolated and mimic clinical situation when transplanted into overt diabetic mice and traced for the protective efficacy. However, in clinical, the islet grafts from donors must be transduced first with viral vectors carrying protective genes before they can be transplanted into recipients. This procedure obviates direct contact of delivery vectors with patients. The most adopted vector systems used in animal models are adenovirus, adeno-associated virus (AAV), and lentivirus which could target both dividing and quiescent cells. Though adenovirus efficiently infects islet cells with low MOI and without affecting beta cell function and survival (149), it expresses as episomal form and could not persist for a long time in infected cells. AAV or lentivirus, in contrast, could integrate its genome carrying target gene into host chromosome and prolong the expression time (150-152) without eliciting strong immune response. However, insertional effects pose new biosafety concerns.

Some protective genes such as TRX and Bcl-2 introduced into islet grafts reveal efficient anti-inflammation and are resistant to apoptosis stress (reviewed in 153). Besides, polarized immune response to Th2 might provide significant protection in T1D. It has been shown that islet grafts transduced with lentivirus carrying IL-4 mediated an impressive resistance to insulitis (154). Inhibitory immunomodulatory molecules such as PD-L1 and CTLA-4 should provide more widely application against the overt immune destruction of grafts. Now potential protective genes are proved and gathered through transgenic animal models or islet transplantation assay, and these have shown potential for a new direction in gene therapy.

6. CONCLUSION

The T1D is a progressive autoimmune disease with complex interactions of immune cells and pancreatic islet milieu. Using transgenic animal models, we systemically and clearly dissect the complicated signal pathways and mechanisms such as inflammatory stimuli, death signals, and activation/tolerance imbalance involved in T1D. On the other hand, more approaches about the counter-regulation against the destructive signaling or immune cell differentiation have been explored using these powerful transgenic mouse models (Figure 2). Now facing the most efficient therapy of T1D, islet transplantation, besides advanced skill for isolation of islet grafts and transplantation procedure, using genetically engineered islet cells will provide better opportunities improving survival and maintaining function.

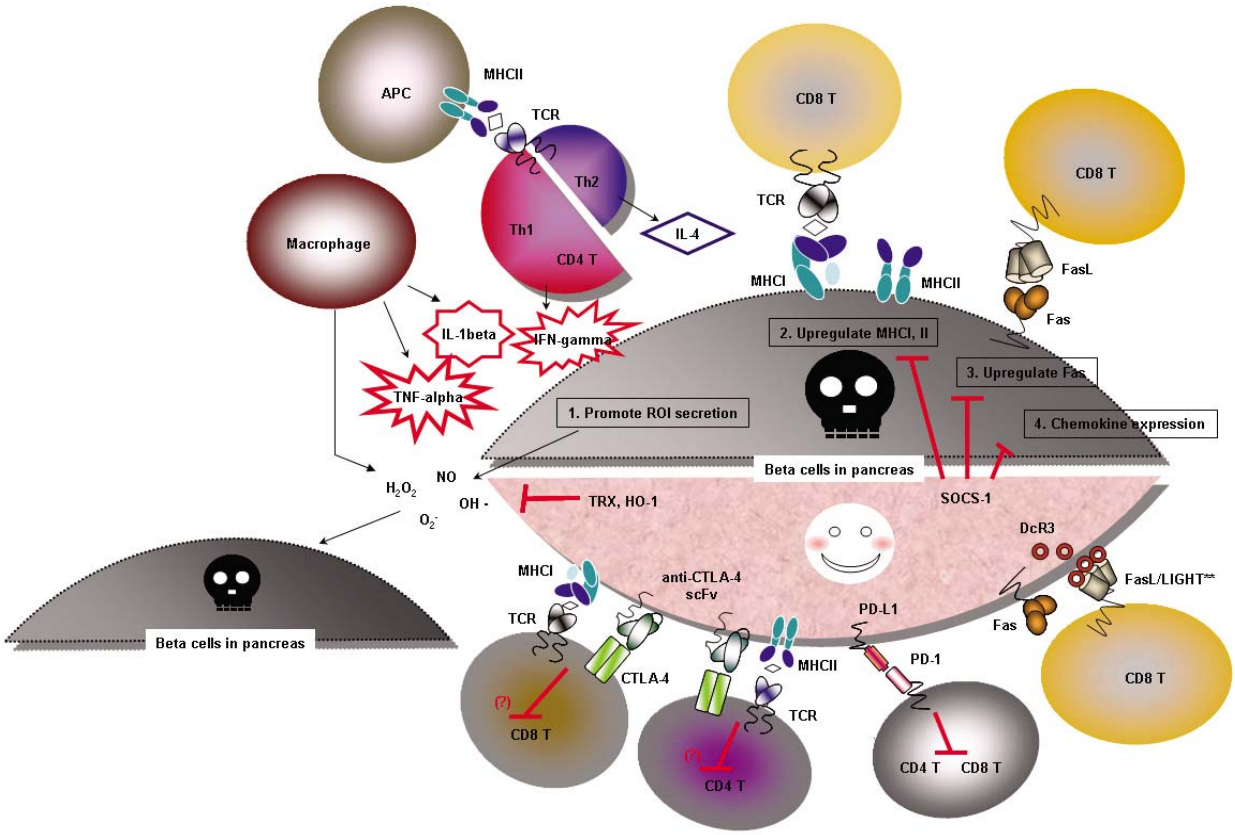

Figure 2. Immunopathogenesis and counter-regulation mechanisms of T1D in islet milieu. Pro-inflammatory cytokines (TNF-alpha, IL-1beta, *et al.*) as well as ROIs, which are mainly produced by macrophages, are the initial mediators resulting in beta cell destruction. Meanwhile, TNF-alpha combined with IFN-gamma secreted by effector Th1 cells triggers: 1. ROIs generation by beta cells; 2. MHC I and II expressions on beta cells; 3. Fas expression on beta cells; 4. Chemokine secretion by beta cells. These molecules manifest CD8 T cells to perform their cytotoxicity against beta cells. Although IL-4 released by Th2 cells counteracts the effect of Th1 cells, the disparity ratio of Th1/Th2 seems no help. Ectopic expression of transgenes restricted in beta cells such as SOCS-1, HO-1, TRX, PD-L1, anti-CTLA-4 scFv, or DcR3, would be beneficial to prevent immune attack mediated by autoreactive T and other inflammatory cells (see details in the text). **LIGHT is expressed on activated T cells.

7. ACKNOWLEDGEMENT

We appreciate the help of L.T. Yeh for scheme preparation; S.J. Shieh and F.C. Chou for insightful discussions. H.K. Sytwu is an investigator of National Defense Medical Center. The research works described in this review were supported by National Science Council (NSC96-3112-B-016-001 to H.-K. Sytwu).

8. REFERENCES

1. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. *Diabetes*, 46, 271-86 (1997)

2. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med*, 329, 977-86 (1993)

3. Wild, S., G. Roglic, A. Green, R. Sicree & H. King: Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27, 1047-53 (2004)

4. Onkamo, P., S. Vaananen, M. Karvonen & J. Tuomilehto: Worldwide increase in incidence of Type I diabetes--the analysis of the data on published incidence trends. *Diabetologia*, 42, 1395-403 (1999)

5. Castano, L. & G. S. Eisenbarth: Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu Rev Immunol*, 8, 647-79 (1990)

6. Barnett, A. H., C. Eff, R. D. Leslie & D. A. Pyke: Diabetes in identical twins. A study of 200 pairs. *Diabetologia*, 20, 87-93 (1981)

7. Smyth, D. J., J. D. Cooper, R. Bailey, S. Field, O. Burren, L. J. Smink, C. Guja, C. Ionescu-Tirgoviste, B. Widmer, D. B. Dunger, D. A. Savage, N. M. Walker, D. G. Clayton & J. A. Todd: A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nat Genet*, 38, 617-9 (2006)

8. Vyse, T. J. & J. A. Todd: Genetic analysis of autoimmune disease. *Cell*, 85, 311-8 (1996)
9. Todd, J. A., N. M. Walker, J. D. Cooper, D. J. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. F. Field, F. Payne, C. E. Lowe, J. S. Szeszko, J. P. Hafler, L. Zeitels, J. H. Yang, A. Vella, S. Nutland, H. E. Stevens, H. Schuilenburg, G. Coleman, M. Maisuria, W. Meadows, L. J. Smink, B. Healy, O. S. Burren, A. A. Lam, N. R. Ovington, J. Allen, E. Adlem, H. T. Leung, C. Wallace, J. M. Howson, C. Guja, C. Ionescu-Tirgoviste, M. J. Simmonds, J. M. Heward, S. C. Gough, D. B. Dunger, L. S. Wicker & D. G. Clayton: Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet*, 39, 857-64 (2007)
10. Todd, J. A., J. I. Bell & H. O. McDevitt: HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature*, 329, 599-604 (1987)
11. Kawabata, Y., H. Ikegami, Y. Kawaguchi, T. Fujisawa, M. Shintani, M. Ono, M. Nishino, Y. Uchigata, I. Lee & T. Ogihara: Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and -DQ haplotypes to susceptibility to type 1 diabetes. *Diabetes*, 51, 545-51 (2002)
12. Rammensee, H. G., T. Friede & S. Stevanoviic: MHC ligands and peptide motifs: first listing. *Immunogenetics*, 41, 178-228 (1995)
13. Atkinson, M. A. & N. K. Maclaren: Islet cell autoantigens in insulin-dependent diabetes. *J Clin Invest*, 92, 1608-16 (1993)
14. Makino, S., K. Kunimoto, Y. Muraoka, Y. Mizushima, K. Katagiri & Y. Tochino: Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu*, 29, 1-13 (1980)
15. Kikutani, H. & S. Makino: The murine autoimmune diabetes model: NOD and related strains. *Adv Immunol*, 51, 285-322 (1992)
16. Delovitch, T. L. & B. Singh: The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. *Immunity*, 7, 727-38 (1997)
17. Bach, J. F. & D. Mathis: The NOD mouse. *Res Immunol*, 148, 285-6 (1997)
18. Christianson, S. W., L. D. Shultz & E. H. Leiter: Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes*, 42, 44-55 (1993)
19. Allison, J., L. C. Harrison, I. L. Campbell & J. F. Miller: Major histocompatibility complex molecules and the beta cell: inferences from transgenic models. *Curr Top Microbiol Immunol*, 156, 121-35 (1990)
20. Sarvetnick, N., D. Liggitt, S. L. Pitts, S. E. Hansen & T. A. Stewart: Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. *Cell*, 52, 773-82 (1988)
21. Severinsson, L. & P. A. Peterson: Beta 2-microglobulin induces intracellular transport of human class I transplantation antigen heavy chains in Xenopus laevis oocytes. *J Cell Biol*, 99, 226-32 (1984)
22. Gagnerault, M. C., J. J. Luan, C. Lotton & F. Lepault: Pancreatic lymph nodes are required for priming of beta cell reactive T cells in NOD mice. *J Exp Med*, 196, 369-77 (2002)
23. Zhang, Y., B. O'Brien, J. Trudeau, R. Tan, P. Santamaria & J. P. Dutz: *In situ* beta cell death promotes priming of diabetogenic CD8 T lymphocytes. *J Immunol*, 168, 1466-72 (2002)
24. Hoglund, P., J. Mintern, C. Waltzinger, W. Heath, C. Benoist & D. Mathis: Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. *J Exp Med*, 189, 331-9 (1999)
25. Trudeau, J. D., J. P. Dutz, E. Arany, D. J. Hill, W. E. Fieldus & D. T. Finegood: Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? *Diabetes*, 49, 1-7 (2000)
26. Yamanouchi, J., J. Verdaguer, B. Han, A. Amrani, P. Serra & P. Santamaria: Cross-priming of diabetogenic T cells dissociated from CTL-induced shedding of beta cell autoantigens. *J Immunol*, 171, 6900-9 (2003)
27. Gordon, J. W. & F. H. Ruddle: Gene transfer into mouse embryos: production of transgenic mice by pronuclear injection. *Methods Enzymol*, 101, 411-33 (1983)
28. Wilkie, T. M., R. L. Brinster & R. D. Palmiter: Germline and somatic mosaicism in transgenic mice. *Dev Biol*, 118, 9-18 (1986)
29. Palmiter, R. D. & R. L. Brinster: Germ-line transformation of mice. *Annu Rev Genet*, 20, 465-99 (1986)
30. Palmiter, R. D., H. Y. Chen & R. L. Brinster: Differential regulation of metallothionein-thymidine kinase fusion genes in transgenic mice and their offspring. *Cell*, 29, 701-10 (1982)
31. Melloul, D., S. Marshak & E. Cerasi: Regulation of insulin gene transcription. *Diabetologia*, 45, 309-26 (2002)
32. Hay, C. W. & K. Docherty: Comparative analysis of insulin gene promoters: implications for diabetes research. *Diabetes*, 55, 3201-13 (2006)
33. Suk, K., S. Kim, Y. H. Kim, K. A. Kim, I. Chang, H. Yagita, M. Shong & M. S. Lee: IFN-gamma/TNF-alpha synergism as the final effector in autoimmune diabetes: a key role for STAT1/IFN regulatory factor-1 pathway in pancreatic beta cell death. *J Immunol*, 166, 4481-9 (2001)
34. Pakala, S. V., M. Chivetta, C. B. Kelly & J. D. Katz: In autoimmune diabetes the transition from benign to pernicious insulitis requires an islet cell response to tumor necrosis factor alpha. *J Exp Med*, 189, 1053-62 (1999)
35. Kagi, D., A. Ho, B. Odermatt, A. Zakarian, P. S. Ohashi & T. W. Mak: TNF receptor 1-dependent beta cell toxicity as an effector pathway in autoimmune diabetes. *J Immunol*, 162, 4598-605 (1999)
36. Cantor, J. & K. Haskins: Effector function of diabetogenic CD4 Th1 T cell clones: a central role for TNF-alpha. *J Immunol*, 175, 7738-45 (2005)
37. Mathis, D., L. Vence & C. Benoist: beta-Cell death during progression to diabetes. *Nature*, 414, 792-8 (2001)
38. Eizirik, D. L. & T. Mandrup-Poulsen: A choice of death--the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia*, 44, 2115-33 (2001)
39. Chong, M. M., H. E. Thomas & T. W. Kay: gamma-Interferon signaling in pancreatic beta-cells is persistent but can be terminated by overexpression of suppressor of cytokine signaling-1. *Diabetes*, 50, 2744-51 (2001)
40. Thomas, H. E., J. L. Parker, R. D. Schreiber & T. W. Kay: IFN-gamma action on pancreatic beta cells causes class I MHC upregulation but not diabetes. *J Clin Invest*, 102, 1249-57 (1998)

41. Kim, K. A., S. Kim, I. Chang, G. S. Kim, Y. K. Min, M. K. Lee, K. W. Kim & M. S. Lee: IFN gamma/TNF alpha synergism in MHC class II induction: effect of nicotinamide on MHC class II expression but not on islet-cell apoptosis. *Diabetologia*, 45, 385-93 (2002)
42. Campbell, I. L., L. Oxbrow, M. Koulmanda & L. C. Harrison: IFN-gamma induces islet cell MHC antigens and enhances autoimmune, streptozotocin-induced diabetes in the mouse. *J Immunol*, 140, 1111-6 (1988)
43. Thomas, H. E., R. Darwiche, J. A. Corbett & T. W. Kay: Evidence that beta cell death in the nonobese diabetic mouse is Fas independent. *J Immunol*, 163, 1562-9 (1999)
44. Yamada, K., N. Takane-Gyotoku, X. Yuan, F. Ichikawa, C. Inada & K. Nonaka: Mouse islet cell lysis mediated by interleukin-1-induced Fas. *Diabetologia*, 39, 1306-12 (1996)
45. Rabinovitch, A., W. L. Suarez-Pinzon, O. Sorensen & R. C. Bleackley: Inducible nitric oxide synthase (iNOS) in pancreatic islets of nonobese diabetic mice: identification of iNOS- expressing cells and relationships to cytokines expressed in the islets. *Endocrinology*, 137, 2093-9 (1996)
46. Mandrup-Poulsen, T.: The role of interleukin-1 in the pathogenesis of IDDM. *Diabetologia*, 39, 1005-29 (1996)
47. Eizirik, D. L., M. Flodstrom, A. E. Karlsen & N. Welsh: The harmony of the spheres: inducible nitric oxide synthase and related genes in pancreatic beta cells. *Diabetologia*, 39, 875-90 (1996)
48. Corbett, J. A., J. L. Wang, M. A. Sweetland, J. R. Lancaster, Jr. & M. L. McDaniel: Interleukin 1 beta induces the formation of nitric oxide by beta-cells purified from rodent islets of Langerhans. Evidence for the beta-cell as a source and site of action of nitric oxide. *J Clin Invest*, 90, 2384-91 (1992)
49. O'Brien, B. A., B. V. Harmon, D. P. Cameron & D. J. Allan: Apoptosis is the mode of beta-cell death responsible for the development of IDDM in the nonobese diabetic (NOD) mouse. *Diabetes*, 46, 750-7 (1997)
50. Kurrer, M. O., S. V. Pakala, H. L. Hanson & J. D. Katz: Beta cell apoptosis in T cell-mediated autoimmune diabetes. *Proc Natl Acad Sci U S A*, 94, 213-8 (1997)
51. Itoh, N., A. Imagawa, T. Hanafusa, M. Waguri, K. Yamamoto, H. Iwahashi, M. Moriwaki, H. Nakajima, J. Miyagawa, M. Namba, S. Makino, S. Nagata, N. Kono & Y. Matsuzawa: Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. *J Exp Med*, 186, 613-8 (1997)
52. Chervonsky, A. V., Y. Wang, F. S. Wong, I. Visintin, R. A. Flavell, C. A. Janeway, Jr. & L. A. Matis: The role of Fas in autoimmune diabetes. *Cell*, 89, 17-24 (1997)
53. Kaneto, H., J. Fujii, H. G. Seo, K. Suzuki, T. Matsuoka, M. Nakamura, H. Tatsumi, Y. Yamasaki, T. Kamada & N. Taniguchi: Apoptotic cell death triggered by nitric oxide in pancreatic beta-cells. *Diabetes*, 44, 733-8 (1995)
54. Held, W., H. R. MacDonald, I. L. Weissman, M. W. Hess & C. Mueller: Genes encoding tumor necrosis factor alpha and granzyme A are expressed during development of autoimmune diabetes. *Proc Natl Acad Sci U S A*, 87, 2239-43 (1990)
55. Higuchi, Y., P. Herrera, P. Muniesa, J. Huarte, D. Belin, P. Ohashi, P. Aichele, L. Orci, J. D. Vassalli & P. Vassalli: Expression of a tumor necrosis factor alpha transgene in murine pancreatic beta cells results in severe and permanent insulitis without evolution towards diabetes. *J Exp Med*, 176, 1719-31 (1992)
56. Picarella, D. E., A. Kratz, C. B. Li, N. H. Ruddle & R. A. Flavell: Transgenic tumor necrosis factor (TNF)-alpha production in pancreatic islets leads to insulitis, not diabetes. Distinct patterns of inflammation in TNF-alpha and TNF-beta transgenic mice. *J Immunol*, 150, 4136-50 (1993)
57. Jacob, C. O., S. Aiso, S. A. Michie, H. O. McDevitt & H. Acha-Orbea: Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): similarities between TNF-alpha and interleukin 1. *Proc Natl Acad Sci U S A*, 87, 968-72 (1990)
58. Green, E. A., E. E. Eynon & R. A. Flavell: Local expression of TNFalpha in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens. *Immunity*, 9, 733-43 (1998)
59. Green, E. A., F. S. Wong, K. Eshima, C. Mora & R. A. Flavell: Neonatal tumor necrosis factor alpha promotes diabetes in nonobese diabetic mice by CD154-independent antigen presentation to CD8 (+) T cells. *J Exp Med*, 191, 225-38 (2000)
60. Lee, L. F., B. Xu, S. A. Michie, G. F. Beilhack, T. Warganich, S. Turley & H. O. McDevitt: The role of TNF-alpha in the pathogenesis of type 1 diabetes in the nonobese diabetic mouse: analysis of dendritic cell maturation. *Proc Natl Acad Sci U S A*, 102, 15995-6000 (2005)
61. Yang, X. D., R. Tisch, S. M. Singer, Z. A. Cao, R. S. Liblau, R. D. Schreiber & H. O. McDevitt: Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med*, 180, 995-1004 (1994)
62. Grewal, I. S., K. D. Grewal, F. S. Wong, D. E. Picarella, C. A. Janeway, Jr. & R. A. Flavell: Local expression of transgene encoded TNF alpha in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J Exp Med*, 184, 1963-74 (1996)
63. Boehm, U., T. Klamp, M. Groot & J. C. Howard: Cellular responses to interferon-gamma. *Annu Rev Immunol*, 15, 749-95 (1997)
64. Suarez-Pinzon, W., R. V. Rajotte, T. R. Mosmann & A. Rabinovitch: Both CD4+ and CD8+ T-cells in syngeneic islet grafts in NOD mice produce interferon-gamma during beta-cell destruction. *Diabetes*, 45, 1350-7 (1996)
65. Sarvetnick, N., J. Shizuru, D. Liggitt, L. Martin, B. McIntyre, A. Gregory, T. Parslow & T. Stewart: Loss of pancreatic islet tolerance induced by beta-cell expression of interferon-gamma. *Nature*, 346, 844-7 (1990)
66. Debray-Sachs, M., C. Carnaud, C. Boitard, H. Cohen, I. Gresser, P. Bedossa & J. F. Bach: Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J Autoimmun*, 4, 237-48 (1991)
67. van den Elsen, P. J., S. J. Gobin, M. C. van Eggermond & A. Peijnenburg: Regulation of MHC class I and II gene transcription: differences and similarities. *Immunogenetics*, 48, 208-21 (1998)
68. Luthman, M. & A. Holmgren: Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry*, 21, 6628-33 (1982)

69. Nakamura, H., K. Nakamura & J. Yodoi: Redox regulation of cellular activation. *Annu Rev Immunol*, 15, 351-69 (1997)
70. Holmgren, A.: Thioredoxin. *Annu Rev Biochem*, 54, 237-71 (1985)
71. Hotta, M., F. Tashiro, H. Ikegami, H. Niwa, T. Ogihara, J. Yodoi & J. Miyazaki: Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J Exp Med*, 188, 1445-51 (1998)
72. Ryter, S. W. & R. M. Tyrrell: The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. *Free Radic Biol Med*, 28, 289-309 (2000)
73. Ryter, S. W., J. Alam & A. M. Choi: Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev*, 86, 583-650 (2006)
74. Li, M., S. Peterson, D. Husney, M. Inaba, K. Guo, A. Kappas, S. Ikehara & N. G. Abraham: Long-lasting expression of HO-1 delays progression of type I diabetes in NOD mice. *Cell Cycle*, 6, 567-71 (2007)
75. Hu, C. M., H. H. Lin, M. T. Chiang, P. F. Chang & L. Y. Chau: Systemic expression of heme oxygenase-1 ameliorates type 1 diabetes in NOD mice. *Diabetes*, 56, 1240-7 (2007)
76. Chong, M. M., H. E. Thomas & T. W. Kay: Suppressor of cytokine signaling-1 regulates the sensitivity of pancreatic beta cells to tumor necrosis factor. *J Biol Chem*, 277, 27945-52 (2002)
77. Frigerio, S., T. Junt, B. Lu, C. Gerard, U. Zumsteg, G. A. Hollander & L. Piali: Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat Med*, 8, 1414-20 (2002)
78. Cardozo, A. K., H. Heimberg, Y. Heremans, R. Leeman, B. Kutlu, M. Kruhoffer, T. Orntoft & D. L. Eizirik: A comprehensive analysis of cytokine-induced and nuclear factor-kappa B-dependent genes in primary rat pancreatic beta-cells. *J Biol Chem*, 276, 48879-86 (2001)
79. Endo, T. A., M. Masuhara, M. Yokouchi, R. Suzuki, H. Sakamoto, K. Mitsui, A. Matsumoto, S. Tanimura, M. Ohtsubo, H. Misawa, T. Miyazaki, N. Leonor, T. Taniguchi, T. Fujita, Y. Kanakura, S. Komiya & A. Yoshimura: A new protein containing an SH2 domain that inhibits JAK kinases. *Nature*, 387, 921-4 (1997)
80. Chong, M. M., Y. Chen, R. Darwiche, N. L. Dudek, W. Irawaty, P. Santamaria, J. Allison, T. W. Kay & H. E. Thomas: Suppressor of cytokine signaling-1 overexpression protects pancreatic beta cells from CD8+ T cell-mediated autoimmune destruction. *J Immunol*, 172, 5714-21 (2004)
81. Rabinovitch, A.: Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation? *Diabetes*, 43, 613-21 (1994)
82. Katz, J. D., C. Benoist & D. Mathis: T helper cell subsets in insulin-dependent diabetes. *Science*, 268, 1185-8 (1995)
83. Shimizu, J., O. Kanagawa & E. R. Unanue: Presentation of beta-cell antigens to CD4+ and CD8+ T cells of non-obese diabetic mice. *J Immunol*, 151, 1723-30 (1993)
84. Haskins, K. & M. McDuffie: Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T cell clone. *Science*, 249, 1433-6 (1990)
85. Rapoport, M. J., A. Jaramillo, D. Zipris, A. H. Lazarus, D. V. Serreze, E. H. Leiter, P. Cyopick, J. S. Danska & T. L. Delovitch: Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J Exp Med*, 178, 87-99 (1993)
86. Pennline, K. J., E. Roque-Gaffney & M. Monahan: Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin Immunol Immunopathol*, 71, 169-75 (1994)
87. Mueller, R., T. Krahl & N. Sarvetnick: Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med*, 184, 1093-9 (1996)
88. Mueller, R., L. M. Bradley, T. Krahl & N. Sarvetnick: Mechanism underlying counterregulation of autoimmune diabetes by IL-4. *Immunity*, 7, 411-8 (1997)
89. Falcone, M., B. Yeung, L. Tucker, E. Rodriguez, T. Krahl & N. Sarvetnick: IL-4 triggers autoimmune diabetes by increasing self-antigen presentation within the pancreatic Islets. *Clin Immunol*, 98, 190-9 (2001)
90. Cardell, S. L.: The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. *Clin Exp Immunol*, 143, 194-202 (2006)
91. You, S., N. Thieblemont, M. A. Alyanakian, J. F. Bach & L. Chatenoud: Transforming growth factor-beta and T-cell-mediated immunoregulation in the control of autoimmune diabetes. *Immunol Rev*, 212, 185-202 (2006)
92. Shull, M. M., I. Ormsby, A. B. Kier, S. Pawlowski, R. J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin & *et al.*: Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature*, 359, 693-9 (1992)
93. Czarniecki, C. W., H. H. Chiu, G. H. Wong, S. M. McCabe & M. A. Palladino: Transforming growth factor-beta 1 modulates the expression of class II histocompatibility antigens on human cells. *J Immunol*, 140, 4217-23 (1988)
94. Zhang, X., L. Giangreco, H. E. Broome, C. M. Dargan & S. L. Swain: Control of CD4 effector fate: transforming growth factor beta 1 and interleukin 2 synergize to prevent apoptosis and promote effector expansion. *J Exp Med*, 182, 699-709 (1995)
95. King, C., J. Davies, R. Mueller, M. S. Lee, T. Krahl, B. Yeung, E. O'Connor & N. Sarvetnick: TGF-beta1 alters APC preference, polarizing islet antigen responses toward a Th2 phenotype. *Immunity*, 8, 601-13 (1998)
96. Peng, Y., Y. Laouar, M. O. Li, E. A. Green & R. A. Flavell: TGF-beta regulates *in vivo* expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. *Proc Natl Acad Sci U S A*, 101, 4572-7 (2004)
97. Andre, I., A. Gonzalez, B. Wang, J. Katz, C. Benoist & D. Mathis: Checkpoints in the progression of autoimmune disease: lessons from diabetes models. *Proc Natl Acad Sci U S A*, 93, 2260-3 (1996)
98. Jarpe, A. J., M. R. Hickman, J. T. Anderson, W. E. Winter & A. B. Peck: Flow cytometric enumeration of mononuclear cell populations infiltrating the islets of

Langerhans in prediabetic NOD mice: development of a model of autoimmune insulitis for type I diabetes. *Reg Immunol*, 3, 305-17 (1990)
99. Miller, B. J., M. C. Appel, J. J. O'Neil & L. S. Wicker: Both the Lyt-2+ and L3T4+ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol*, 140, 52-8 (1988)
100. Bendelac, A., C. Carnaud, C. Boitard & J. F. Bach: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4+ and Lyt-2+ T cells. *J Exp Med*, 166, 823-32 (1987)
101. Bradley, B. J., K. Haskins, F. G. La Rosa & K. J. Lafferty: CD8 T cells are not required for islet destruction induced by a CD4+ islet-specific T-cell clone. *Diabetes*, 41, 1603-8 (1992)
102. Wicker, L. S., E. H. Leiter, J. A. Todd, R. J. Renjilian, E. Peterson, P. A. Fischer, P. L. Podolin, M. Zijlstra, R. Jaenisch & L. B. Peterson: Beta 2-microglobulin-deficient NOD mice do not develop insulitis or diabetes. *Diabetes*, 43, 500-4 (1994)
103. Sumida, T., M. Furukawa, A. Sakamoto, T. Namekawa, T. Maeda, M. Zijlstra, I. Iwamoto, T. Koike, S. Yoshida, H. Tomioka & *et al.*: Prevention of insulitis and diabetes in beta 2-microglobulin-deficient non-obese diabetic mice. *Int Immunol*, 6, 1445-9 (1994)
104. Serreze, D. V., E. H. Leiter, G. J. Christianson, D. Greiner & D. C. Roopenian: Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes and insulitis resistant. *Diabetes*, 43, 505-9 (1994)
105. Katz, J., C. Benoist & D. Mathis: Major histocompatibility complex class I molecules are required for the development of insulitis in non-obese diabetic mice. *Eur J Immunol*, 23, 3358-60 (1993)
106. Efrat, S., D. Serreze, A. Svetlanov, C. M. Post, E. A. Johnson, K. Herold & M. Horwitz: Adenovirus early region 3 (E3) immunomodulatory genes decrease the incidence of autoimmune diabetes in NOD mice. *Diabetes*, 50, 980-4 (2001)
107. Kagi, D., B. Odermatt, P. Seiler, R. M. Zinkernagel, T. W. Mak & H. Hengartner: Reduced incidence and delayed onset of diabetes in perforin-deficient nonobese diabetic mice. *J Exp Med*, 186, 989-97 (1997)
108. Wong, F. S., I. Visintin, L. Wen, J. Granata, R. Flavell & C. A. Janeway: The role of lymphocyte subsets in accelerated diabetes in nonobese diabetic-rat insulin promoter-B7-1 (NOD-RIP-B7-1) mice. *J Exp Med*, 187, 1985-93 (1998)
109. Wong, F. S., W. Du, I. J. Thomas & L. Wen: The influence of the major histocompatibility complex on development of autoimmune diabetes in RIP-B7.1 mice. *Diabetes*, 54, 2032-40 (2005)
110. Stephens, L. A. & T. W. Kay: Pancreatic expression of B7 co-stimulatory molecules in the non-obese diabetic mouse. *Int Immunol*, 7, 1885-95 (1995)
111. Sharpe, A. H., E. J. Wherry, R. Ahmed & G. J. Freeman: The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol*, 8, 239-45 (2007)
112. Greenwald, R. J., G. J. Freeman & A. H. Sharpe: The B7 family revisited. *Annu Rev Immunol*, 23, 515-48 (2005)
113. Khoury, S. J. & M. H. Sayegh: The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity*, 20, 529-38 (2004)
114. Dong, C., R. I. Nurieva & D. V. Prasad: Immune regulation by novel costimulatory molecules. *Immunol Res*, 28, 39-48 (2003)
115. Fife, B. T., I. Guleria, M. Gubbels Bupp, T. N. Eagar, Q. Tang, H. Bour-Jordan, H. Yagita, M. Azuma, M. H. Sayegh & J. A. Bluestone: Insulin-induced remission in new-onset NOD mice is maintained by the PD-1-PD-L1 pathway. *J Exp Med*, 203, 2737-47 (2006)
116. Keir, M. E., S. C. Liang, I. Guleria, Y. E. Latchman, A. Qipo, L. A. Albacker, M. Koulmanda, G. J. Freeman, M. H. Sayegh & A. H. Sharpe: Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med*, 203, 883-95 (2006)
117. Sperling, A. I. & J. A. Bluestone: The complexities of T-cell co-stimulation: CD28 and beyond. *Immunol Rev*, 153, 155-82 (1996)
118. Krummel, M. F. & J. P. Allison: CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. *J Exp Med*, 183, 2533-40 (1996)
119. Krummel, M. F. & J. P. Allison: CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*, 182, 459-65 (1995)
120. Eagar, T. N., N. J. Karandikar, J. A. Bluestone & S. D. Miller: The role of CTLA-4 in induction and maintenance of peripheral T cell tolerance. *Eur J Immunol*, 32, 972-81 (2002)
121. Perez, V. L., L. Van Parijs, A. Biuckians, X. X. Zheng, T. B. Strom & A. K. Abbas: Induction of peripheral T cell tolerance *in vivo* requires CTLA-4 engagement. *Immunity*, 6, 411-7 (1997)
122. Luhder, F., P. Hoglund, J. P. Allison, C. Benoist & D. Mathis: Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) regulates the unfolding of autoimmune diabetes. *J Exp Med*, 187, 427-32 (1998)
123. Lin, H., J. C. Rathmell, G. S. Gray, C. B. Thompson, J. M. Leiden & M. L. Alegre: Cytotoxic T lymphocyte antigen 4 (CTLA4) blockade accelerates the acute rejection of cardiac allografts in CD28-deficient mice: CTLA4 can function independently of CD28. *J Exp Med*, 188, 199-204 (1998)
124. McCoy, K., M. Camberis & G. L. Gros: Protective immunity to nematode infection is induced by CTLA-4 blockade. *J Exp Med*, 186, 183-7 (1997)
125. Leach, D. R., M. F. Krummel & J. P. Allison: Enhancement of antitumor immunity by CTLA-4 blockade. *Science*, 271, 1734-6 (1996)
126. Karandikar, N. J., C. L. Vanderlugt, T. L. Walunas, S. D. Miller & J. A. Bluestone: CTLA-4: a negative regulator of autoimmune disease. *J Exp Med*, 184, 783-8 (1996)
127. Griffin, M. D., D. K. Hong, P. O. Holman, K. M. Lee, M. J. Whitters, S. M. O'Herrin, F. Fallarino, M. Collins, D. M. Segal, T. F. Gajewski, D. M. Kranz & J. A. Bluestone: Blockade of T cell activation using a surface-linked single-chain antibody to CTLA-4 (CD152). *J Immunol*, 164, 4433-42 (2000)
128. Fife, B. T., M. D. Griffin, A. K. Abbas, R. M. Locksley & J. A. Bluestone: Inhibition of T cell activation

and autoimmune diabetes using a B cell surface-linked CTLA-4 agonist. *J Clin Invest*, 116, 2252-61 (2006)
129. Mackay, F. & S. L. Kalled: TNF ligands and receptors in autoimmunity: an update. *Curr Opin Immunol*, 14, 783-90 (2002)
130. Silva, D. G., L. Socha, B. Charlton, W. Cowden & N. Petrovsky: Autoimmune diabetes in the NOD mouse: an essential role of Fas-FasL signaling in beta cell apoptosis. *Ann N Y Acad Sci*, 1005, 161-5 (2003)
131. Suarez-Pinzon, W., O. Sorensen, R. C. Bleackley, J. F. Elliott, R. V. Rajotte & A. Rabinovitch: Beta-cell destruction in NOD mice correlates with Fas (CD95) expression on beta-cells and proinflammatory cytokine expression in islets. *Diabetes*, 48, 21-8 (1999)
132. Stassi, G., R. De Maria, G. Trucco, W. Rudert, R. Testi, A. Galluzzo, C. Giordano & M. Trucco: Nitric oxide primes pancreatic beta cells for Fas-mediated destruction in insulin-dependent diabetes mellitus. *J Exp Med*, 186, 1193-200 (1997)
133. Sung, H. H., J. H. Juang, Y. C. Lin, C. H. Kuo, J. T. Hung, A. Chen, D. M. Chang, S. Y. Chang, S. L. Hsieh & H. K. Sytwu: Transgenic expression of decoy receptor 3 protects islets from spontaneous and chemical-induced autoimmune destruction in nonobese diabetic mice. *J Exp Med*, 199, 1143-51 (2004)
134. Pitti, R. M., S. A. Marsters, D. A. Lawrence, M. Roy, F. C. Kischkel, P. Dowd, A. Huang, C. J. Donahue, S. W. Sherwood, D. T. Baldwin, P. J. Godowski, W. I. Wood, A. L. Gurney, K. J. Hillan, R. L. Cohen, A. D. Goddard, D. Botstein & A. Ashkenazi: Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. *Nature*, 396, 699-703 (1998)
135. Yu, K. Y., B. Kwon, J. Ni, Y. Zhai, R. Ebner & B. S. Kwon: A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. *J Biol Chem*, 274, 13733-6 (1999)
136. Migone, T. S., J. Zhang, X. Luo, L. Zhuang, C. Chen, B. Hu, J. S. Hong, J. W. Perry, S. F. Chen, J. X. Zhou, Y. H. Cho, S. Ullrich, P. Kanakaraj, J. Carrell, E. Boyd, H. S. Olsen, G. Hu, L. Pukac, D. Liu, J. Ni, S. Kim, R. Gentz, P. Feng, P. A. Moore, S. M. Ruben & P. Wei: TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. *Immunity*, 16, 479-92 (2002)
137. Savinov, A. Y., A. Tcherepanov, E. A. Green, R. A. Flavell & A. V. Chervonsky: Contribution of Fas to diabetes development. *Proc Natl Acad Sci U S A*, 100, 628-32 (2003)
138. Wang, J., J. C. Lo, A. Foster, P. Yu, H. M. Chen, Y. Wang, K. Tamada, L. Chen & Y. X. Fu: The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. *J Clin Invest*, 108, 1771-80 (2001)
139. DeBenedette, M. A., A. Shahinian, T. W. Mak & T. H. Watts: Costimulation of CD28- T lymphocytes by 4-1BB ligand. *J Immunol*, 158, 551-9 (1997)
140. Mittler, R. S., T. S. Bailey, K. Klussman, M. D. Trailsmith & M. K. Hoffmann: Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses *in vivo* through the induction of helper T cell anergy. *J Exp Med*, 190, 1535-40 (1999)
141. Sun, Y., X. Lin, H. M. Chen, Q. Wu, S. K. Subudhi, L. Chen & Y. X. Fu: Administration of agonistic anti-4-1BB monoclonal antibody leads to the amelioration of experimental autoimmune encephalomyelitis. *J Immunol*, 168, 1457-65 (2002)
142. Sytwu, H. K., W. D. Lin, S. R. Roffler, J. T. Hung, H. S. Sung, C. H. Wang, T. L. Cheng, S. C. Tsou, S. C. Hsi & K. L. Shen: Anti-4-1BB-based immunotherapy for autoimmune diabetes: lessons from a transgenic non-obese diabetic (NOD) model. *J Autoimmun*, 21, 247-54 (2003)
143. Shapiro, A. M., C. Ricordi, B. J. Hering, H. Auchincloss, R. Lindblad, R. P. Robertson, A. Secchi, M. D. Brendel, T. Berney, D. C. Brennan, E. Cagliero, R. Alejandro, E. A. Ryan, B. DiMercurio, P. Morel, K. S. Polonsky, J. A. Reems, R. G. Bretzel, F. Bertuzzi, T. Froud, R. Kandaswamy, D. E. Sutherland, G. Eisenbarth, M. Segal, J. Preiksaitis, G. S. Korbutt, F. B. Barton, L. Viviano, V. Seyfert-Margolis, J. Bluestone & J. R. Lakey: International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*, 355, 1318-30 (2006)
144. Ryan, E. A., B. W. Paty, P. A. Senior, D. Bigam, E. Alfadhli, N. M. Kneteman, J. R. Lakey & A. M. Shapiro: Five-year follow-up after clinical islet transplantation. *Diabetes*, 54, 2060-9 (2005)
145. Biarnes, M., M. Montolio, V. Nacher, M. Raurell, J. Soler & E. Montanya: Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes*, 51, 66-72 (2002)
146. Davalli, A. M., Y. Ogawa, C. Ricordi, D. W. Scharp, S. Bonner-Weir & G. C. Weir: A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation*, 59, 817-20 (1995)
147. Ozmen, L., K. N. Ekdahl, G. Elgue, R. Larsson, O. Korsgren & B. Nilsson: Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes*, 51, 1779-84 (2002)
148. Moberg, L., H. Johansson, A. Lukinius, C. Berne, A. Foss, R. Kallen, O. Ostraat, K. Salmela, A. Tibell, G. Tufveson, G. Elgue, K. Nilsson Ekdahl, O. Korsgren & B. Nilsson: Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*, 360, 2039-45 (2002)
149. Weber, M., S. Deng, T. Kucher, A. Shaked, R. J. Ketchum & K. L. Brayman: Adenoviral transfection of isolated pancreatic islets: a study of programmed cell death (apoptosis) and islet function. *J Surg Res*, 69, 23-32 (1997)
150. Loiler, S. A., T. J. Conlon, S. Song, Q. Tang, K. H. Warrington, A. Agarwal, M. Kapturczak, C. Li, C. Ricordi, M. A. Atkinson, N. Muzyczka & T. R. Flotte: Targeting recombinant adeno-associated virus vectors to enhance gene transfer to pancreatic islets and liver. *Gene Ther*, 10, 1551-8 (2003)
151. Zufferey, R., D. Nagy, R. J. Mandel, L. Naldini & D. Trono: Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat Biotechnol*, 15, 871-5 (1997)
152. Naldini, L., U. Blomer, F. H. Gage, D. Trono & I. M. Verma: Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci U S A*, 93, 11382-8 (1996)

153. Bottino, R., P. Lemarchand, M. Trucco & N. Giannoukakis: Gene- and cell-based therapeutics for type I diabetes mellitus. *Gene Ther*, 10, 875-89 (2003)
154. Gallichan, W. S., T. Kafri, T. Krahl, I. M. Verma & N. Sarvetnick: Lentivirus-mediated transduction of islet grafts with interleukin 4 results in sustained gene expression and protection from insulitis. *Hum Gene Ther*, 9, 2717-26 (1998)
155. Allison, J., I. L. Campbell, G. Morahan, T. E. Mandel, L. C. Harrison & J. F. Miller: Diabetes in transgenic mice resulting from over-expression of class I histocompatibility molecules in pancreatic beta cells. *Nature*, 333, 529-33 (1988)
156. Lo, D., L. C. Burkly, G. Widera, C. Cowing, R. A. Flavell, R. D. Palmiter & R. L. Brinster: Diabetes and tolerance in transgenic mice expressing class II MHC molecules in pancreatic beta cells. *Cell*, 53, 159-68 (1988)

Key Words: T1D, IDDM, NOD, Insulin Promoter, Immunopathogenesis, Islet Transplantation, Review

Send correspondence to: Huey-Kang, Sytwu; Address: No.161, Section 6, Min-Chuan East Road, Taipei 114, Taiwan, ROC. Tel: 886-2-87923100 ext 18539, Fax: 886-2-87921774, E-mail: sytwu@ndmctsgh.edu.tw

http://www.bioscience.org/current/vol13.htm