Fas/Fas-Ligand Interaction As a Mechanism of Immune Homeostasis and β-Cell Cytotoxicity: Enforcement Rather Than Neutralization for Treatment of Type 1 Diabetes

Esma S. Yolcu¹, Haval Shirwan¹ and Nadir Askenasy²*

¹ Department of Microbiology and Immunology, Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, USA, ² Frankel Laboratory of Experimental Bone Marrow Transplantation, Petach Tikva, Israel

Keywords: type 1 diabetes, β-cell inflammation, activation-induced cell death, Fas, Fas-ligand

INTRODUCTION

Receptor/ligand interactions of the tumor necrosis factor (TNF) superfamily are associated with versatile apoptotic and regulatory signaling pathways in parenchymal tissues and the immunohematopoietic system. Apoptotic signaling mediated by Fas and TNF receptor-1 (TNF-R1) is one of the major cytotoxic mechanisms used by immune cells to kill endogenous cells and exogenous pathogens. In this capacity, these ubiquitous effector mechanisms of cell death, along with perforin/granzyme, are direct mediators of β-cell injury that is inflicted in autoimmune insulitis in type 1 diabetes (T1D). At the same time, the TNF family receptor/ligand interactions are prime constituents of immune homeostasis that enforce negative regulation of sensitized immunocytes (1–3). All immune cells upregulate TNF family receptors upon activation and are therefore submitted to negative regulation by apoptosis within the process of activation-induced cell death (AICD), which is also essential to termination of inflammation (4).

The Fas/FasL interaction is a common effector mechanism of β-cell apoptosis and islet injury under inflammatory conditions, and physiological modulation of immune homeostasis, including control of aberrant autoimmune reactions. In the absence of specific characteristics of the toxic and anti-inflammatory pathways, it is questioned whether neutralization or reinforcement of the Fas/FasL interaction is of therapeutic value in autoimmune disorders. We examine a wide array of evidence arguing in favor of and against therapeutic neutralization of Fas and/or FasL and conversely, consider the feasibility of implementation of this molecular interaction as approaches to abrogate
autoimmune diabetes. Despite focus on a particular signaling pathway, the Fas/Fasl interaction, in a particular autoimmune disorder, T1D, the debate is rather relevant to multiple receptor/ligand interactions of the TNF superfamily and to the entire range of inflammatory and autoimmune disorders.

**WHY FOCUS ON THE Fas/FasL INTERACTION?**

The Fas/FasL interaction attracts attention as the common executioner of apoptosis in the TNF superfamily endowed with distinct characteristics (10). Like perforin/granzyme (11, 12), apoptotic signaling by Fas/Fasl is localized by the requirement of direct contact between the effector and target cells, a unique feature caused by essential Fas receptor trimerization through engagement of the membrane-bound or oligomers of the ligand. The physiological significance of this receptor/ligand interaction is emphasized by lymphoproliferative disorders resulting from disruption of homeostatic negative regulation in Fas-deficient (lpr) and Fasl-defective (gld) mice (13). Despite these particular characteristics of the Fas/Fasl interaction, common physiological trophic and apoptotic activities are shared by soluble ligands of the TNF superfamily, including TNFα and TNF-related apoptosis-inducing ligand receptor-1 (8).

**PHYSIOLOGICAL ACTIVITIES OF THE Fas/ Fasl INTERACTION IN PANCREATIC ISLETS**

**Non-Immuneogenic Activities**

Several components of the signaling pathways associated with the Fas receptor are involved in insulin secretion (14, 15) and physiological adjustment of β-cell mass. For example, the antia apoptotic factor FADD-like interleukin-1β-converting enzyme-inhibitory protein acting as a competitive caspase-8 antagonist promotes β-cell growth (16) under the inductive influence of interleukin-1β (IL-1β) (17). The Fas signaling pathway is also involved in regulation of insulin secretion (14, 15).

**Immuneogenic Activities**

Physiological immune privilege uses Fasl to defend organs from excessive inflammation that is more harmful than pathogens, such as the anterior eye chamber and reproductive organs (10, 18). The pancreas is not one of the first line immune privileged tissues. However, both islet cells and vascular endothelium constitutively express Fasl, while the Fas receptor is prevalently detected in resident macrophages (19, 20) and in inflamed islets (21, 22). The histological pattern of expression is suggestive of a defensive rim of Fasl-expressing α- and β-cells in the islets of Langerhans (23). In fact, the frontier battleground between reactive T cells and tissues is islet vasculature (24), which constitutively expresses both Fas and Fasl (25) and is therefore inherently insensitive to Fas-mediated apoptosis (26, 27). Protection of the islets by relative immune privilege represents a wider network of defense of the pancreas from incidental inflammation and immune attack (28) due to the extensive digestive capacity of pancreatic enzymes responsible for intestinal degradation of substrates, which might cause autolytic pancreatitis associated with severe morbidity and mortality.

**THE Fas/FasL INTERACTION IN INFLAMMATORY INSULITIS**

**The Fas/FasL Interaction As a Mechanism of β-Cell Death**

Naïve islets express low levels of Fas (21, 22) and are intrinsically resistant to apoptosis triggered by this receptor (29, 30). Islets become gradually sensitive to apoptosis along the course of inflammation (31–33) due to increased Fas expression in β-cells (34, 35) and concomitant sensitization to Fas-mediated apoptosis (36, 37). Uregulated expression of the Fas receptor is one of the many features of the transcriptional profiles of inflamed islets (38, 39) caused by activation of the nuclear factor-κB (NFkB) pathways (40). Induced Fas transcription and modulation of expression is caused by a number of pro-inflammatory cytokines including IL-1α, IL-1β, IFNγ, nitric oxide (NO), and TNF-α that synergize with Fas as effector mechanisms of β-cell destruction (37, 41). Furthermore, both Fas expression induced by IL-1β (42) independent of NFkB activation (43, 44) and islet sensitization to apoptosis (45, 46) also evolve as direct consequences of hyperglycemia. The vast changes in transcriptomes and expression profiles of the inflamed islets may be viewed as an effort of the tissue to sustain insulin production and increase β-cell mass. However, cytokines secreted by the islets themselves paradoxically enhance immune activation and islet injury under inflammatory conditions (47–49).

**Potential Therapeutic Efficacy of Fas and/ or Fasl Neutralization**

There is extensive evidence emphasizing a pivotal role of the Fas/Fasl interaction in destructive insulitis in T1D, including experiments performed in transgenes deficient in the receptor and/or the ligand. For example, homozygous transgenes of non-obese diabetic (NOD) mice deficient in the Fas receptor (lpr) are protected from spontaneous evolution of diabetes, and heterozygous NOD.lpr transgenes display severe mononuclear infiltration in the islets without hyperglycemia (50, 51). However, NOD.lpr with and without superposed SCID mutations that display reduced incidence of spontaneous diabetes are susceptible to islet injury inflicted by adoptive transfer of diabetogenic cells (52). Likewise, disease incidence is reduced in Fasl-deficient transgenes (gld) crossed onto the NOD background (52, 53). It is logical and tempting to approach inflammatory insulitis by neutralization of the Fas/Fasl interaction as one of the pivotal cytotoxic mechanisms used by diabetogenic effectors to attack β-cells (54–57), similar to the potential therapeutic benefit of TNF-α neutralization (58). However, it should be noted that Fasl neutralization in the early postnatal period (53, 59) and in very early stages of inflammation slowed the pace but failed to prevent evolution of insulitis (60).
ARGUMENTS AGAINST THERAPEUTIC NEUTRALIZATION OF THE Fas/FasL INTERACTION

Multiple Immune Mechanisms Modulate Inflammatory Insulitis in the Absence of Functional Fas/FasL Signaling

The insights into effective disease diversion by interruption of functional Fas/FasL signaling led to identification of a number of indirect immunogenic factors that modulate the course of inflammation in transgenic mice. The variable patterns of disease expression in lpr transgenes are explained by two mechanisms beyond relative insensitivity of Fas-deficient islets to apoptosis. First, slow pace of spontaneous inflammatory insulitis is attributed to reduced aggressiveness and slow proliferation of effector lymphocytes of the lpr transgenes (61). Second, T cells of lpr transgenes overexpress FasL, which inhibits the activity of endogenous and adoptively transferred diabetogenic cells (62). In variance, the pace of disease is slowed in gld transgenes by the activity of B lymphocytes that attenuate the course of inflammation by enhanced secretion of IL-10 (63). It is therefore evident that disruption of a pivotal immune homeostatic mechanism such as the Fas/FasL interaction has quite significant consequences that affect autoimmunity beyond direct participation as a cytotoxic mechanism of islet injury.

Multiple Redundant Mechanisms of β-Cell Death Obviate Fas Neutralization

The difficulty in designation of an exact role of Fas-mediated apoptosis stands in the multiple, redundant, and interrelated mechanisms of β-cell death in T1D. The progressive involvement of Fas as a mediator of apoptosis along the course of inflammation has been challenged by a series of studies showing that this mechanism is neither obligatory nor essential in the process of destructive insulitis (64–68). Although Fas expression correlates with β-cell inflammation and unequivocally contributes to destructive insulitis, a causal relationship is rather complex because apoptosis also correlates with upregulation of granzyme and TNF-R1 (69–72). In addition to the canonical mechanisms of apoptosis, islet injury is inflicted by a number of cytotoxic cytokines such as IL-1β, IFNγ, and NO (73, 74). It is quite difficult to attribute distinct activities to these interrelated mechanisms in the process of inflammatory insulitis, because most cytokines as well as TNFα induce Fas expression (21, 22, 29, 75).

Individual Cytotoxic Mechanisms Are Dispensable in Islet Destruction

Each one of the canonical cytotoxic mechanisms, including Fas, TNF-R1, and perforin/granzyme, is dispensable in autoimmune β-cell destruction (68, 75–80), as well as islet allograft rejection (27, 81). Outstanding is the compensation of dysfunctional Fas/FasL interactions by other effector mechanisms of β-cell death (11, 35, 69, 82–84). Likewise, redundant activity of TGF-β, IL-1β, IFNγ, and NO is a common characteristic, as each individual cytotoxic mechanism is largely dispensable in β-cell lysis (36, 41, 47, 85).

Harnessing Physiological Mechanisms to Counteract Islet Inflammation

Extending the mechanism of immune privilege, negative regulation of immune cells by TNF family receptor/ligand interactions has significant homeostatic impact on the intensity of inflammatory reactions. The common mechanism of tissue defense involves induction of apoptosis in autoreactive effectors sensitive to AICD at the level of pancreatic islets in situ (30, 62, 86). Physical elimination of diabetogenic cells has been attained by targeted expression of TNFα (87–90) and TGFβ (91, 92) under control of the insulin promoter, systemic administration of TNF-α (93–95), and overexpression of FasL protein in regulatory T cells (30, 96).

Therapeutic Implications of the Fas/FasL Interaction

The analysis presented here suggests that inhibition of the Fas/FasL interaction has little potential efficacy in prevention of β-cell death by inflammatory insulitis, while targeted reinforcement of this mechanisms of immune homeostasis holds the potential to abrogate diabetic autoimmunity. Signaling through Fas receptor is one of many redundant and dispensable mechanisms of β-cell lysis by autoimmune attack; thus, neutralization provides transient symptomatic relief with little impact on alternative cytotoxic mechanisms (64–67). Implementation of FasL neutralization for treatment of T1D might even result in increased incidence of malignancies as seen with TNF-α inhibitors (97–99). On the contrary, the role of Fasl along TNF-α and perforin/granzyme is much more significant and non-redundant in immune homeostasis than in induction of β-cell death (10, 61, 62, 88, 100). These homeostatic immune mechanisms counteract inflammation as mediators of effector cell death (27, 30, 86, 89–96) are mandatory to reestablishment of suppressor mechanisms (101) and are indispensable in termination of inflammatory reactions (4).

AUTHOR CONTRIBUTIONS

All authors contributed equally to this manuscript.

FUNDING

No dedicated funding to be declared.

REFERENCES

1. Cohen JJ, Duke RC. Apoptosis and programmed cell death in immunity. Ann Rev Immunol (1992) 10:267–93. doi:10.1146/annurev.immunol.10.1.267
2. Kabelitz D, Pohl T, Pechhold K. Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. Immunol Today (1993) 14:338–9. doi:10.1016/0167-5699(93)90231-9
3. Nagata S, Golstein P. The Fas death factor. Science (1995) 267:1449–56. doi:10.1126/science.7533326
Yolcu ES, Ash S, Kaminitz A, Sagiv T, Askensy N, Yarkoni S. Apoptosis as a mechanism of T-regulatory cell homeostasis and suppression. *Immunol Cell Biol* (2000) 78:95–107. doi:10.1038/icb.2000.62

Desbarats J, Newell MK. Fas engagement accelerates liver regeneration after partial hepatectomy. *Nat Med* (2000) 6:920–3. doi:10.1038/78688

Lambert C, Landau AM, Desbarats J. Fas-beyond death: a regenerative role for Fas in the nervous system. *Apoptosis* (2003) 8:551–62. doi:10.1021/3/A102611322478

Song JH, Bellail A, Tse MC, Yong VW, Hao C. Human astrocytes are resistant to Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. *J Neurosci* (2006) 26:3299–308. doi:10.1523/JNEUROSCI.5572-05.2006

Mizrahi K, Askensy N. Physiological functions of TNF family receptor/ligand interactions in hematopoiesis and transplantation. *Blood* (2014) 124:176–83. doi:10.1182/blood-2013-05-59641

Askensy N. Interferon and tumor necrosis factor as humoral mechanisms coupling hematopoietic activity to inflammation and injury. *Blood Rev* (2015) 29:1–15. doi:10.1016/j.bler.2014.09.002

Askensy N, Yolcu ES, Yaniv I, Shirwan H. Induction of tolerance using Fas ligand in the pancreas. *Diabetes* (1999) 48:3135–41. doi:10.2337/diabetes.48.11.3135

Schumann DM, Maedler K, Franklin I, Konrad D, Størling J, Böni-Schnetzler M, et al. Low concentration of interleukin-1beta induces FLICE-inhibitory protein-mediated beta-cell proliferation in human pancreatic islets. *Diabetes* (2002) 51:8236–41. doi:10.2337/diabetes.51.10.8236

Askenasy N, Yolcu ES, Yaniv I, Shirwan H. T cell-mediated destruction of pancreatic beta-cells and proinflammatory cytokine expression in islets of NOD mice. *J Autoimmun* (1998) 11:231–8. doi:10.1006/jaut.1997.0131

Green DR, Ferguson RA, Hollander GA. Nitric oxide production and Fas surface expression mediate two independent pathways of cytokine-induced murine beta-cell damage. *Diabetes* (2000) 49:39–47. doi:10.2337/diabetes.49.1.39

Suarez-Pinzon W, Sorensen O, Bleackley RC, Elliott JF, Rajotte RV, Rabinovich A. Beta-cell destruction in NOD mice correlates with Fas (CD95) expression on beta-cells and proinflammatory cytokine expression in islets. *Diabetes* (1999) 48:21–8. doi:10.2337/diabetes.48.1.21

Amrani A, Verdaguer J, Thiessen S, Bou S, Santamaria P, Il-1alpha, IL-1beta, and IFN-gamma mark beta cells for Fas-dependent destruction by diabetogenic CD4+ T lymphocytes. *J Clin Invest* (2000) 105:459–68. doi:10.1172/JCI8185

Maedler K, Spinas GA, Lehmann R, Sergeev P, Weber M, Fontana A, et al. Fas and Fas ligand: a double-edged immunomodulator. *Blood* (2005) 105:1396–5. doi:10.1182/blood-2004-09-2203

Fas and Fas ligand expression in inflamed islets in pancreas sections of patients with recent-onset Type I diabetes mellitus. *Diabetologia* (1999) 42:1332–40. doi:10.1002/1097-0012(199910).print

Darwinche R, Chong MM, Santamaria P, Thomas HE, Kay TW. Fas is detectable on beta cells in accelerated, but not spontaneous, diabetes in nonobese diabetic mice. *J Immunol* (2003) 170:2292–7. doi:10.4049/jimmunol.170.12.2292

Rabinovich A. Immunoregulation by cytokines in autoimmune diabetes. *Adv Exp Med Biol* (2003) 520:159–93.

von Herrath M, Holz A. Pathological changes in the islet milieu precede infiltration of islets and destruction of beta-cells by autoreactive lymphocytes in a transgenic model of virus-induced IDDM. *J Autoimmun* (1997) 10:231–8. doi:10.1006/jaut.1997.0131

Aspord C, Rome S, Thivolet C. Early events in islets and pancreatic lymph nodes in autoimmune diabetes. *J Autoimmun* (2004) 23:27–35. doi:10.1016/j.jauto.2004.03.007

Kwon G, Corbett JA, Rodi CP, Sullivan P, McDaniel ML. Interleukin-1beta inhibits interferon-gamma-induced beta-cell death in patients with recent-onset Type I diabetes mellitus. *Diabetes* (2001) 50:1683–90. doi:10.2337/diabetes.50.8.1683
55. Baumann B, Salem HH, Boehm BO. Anti-inflammatory therapy in type 1 diabetes.

57. Sedger LM, McDermott MF. TNF and TNF-receptors: from mediators of cell death and inflammation to therapeutic giants – past, present and future. Cytokine Growth Factor Rev (2014) 24:453–72. doi:10.1016/j.cytogfr.2014.07.016

58. Yang X, Tisch R, Singer SM, Cao ZA, Lihlau RS, Schreiber RD, et al. Effect of tumor necrosis factor [alpha] on insulin-dependent diabetic mellitus in NOD mice. I. The early development of insulin autoimmunity and the diabeticogenic process. J Exp Med (2004) 190:995–1004. doi:10.1084/jem.180.3.995

59. Nakayama M, Nagata M, Yasuda H, Arisawa K, Kotani R, Yamada K, et al. Fas/Fas ligand interactions play an essential role in the initiation of murine autoimmune diabetes. Diabetes (2002) 51:1391–7. doi:10.2337/diabetes.51.11.2797

60. Hamad AR, Arcara K, Uddin S, Donner T. The potential of Fas ligand (apoptosis-inducing molecule) as an unconventional therapeutic target in type 1 diabetes. Front Immunol (2012) 3:196. doi:10.3389/fimmu.2012.00196

61. Vence L, Benoist C, Mathis D. Fas deficiency prevents type 1 diabetes by inducing hypo-responsiveness in islet β-cell reactive T-cells. Diabetes (2004) 53:2797–803. doi:10.2337/diabetes.53.11.2797

62. Kim S, Kim KA, Hwang DY, Lee TH, Kagayaki N, Yagita H, et al. Inhibition of autoimmune diabetes by Fas ligand: the paradox is solved. J Immunol (2000) 164:2931–6. doi:10.4049/jimmunol.164.6.2931
development of spontaneous diabetes in non-obese diabetic mice. *Clin Exp Immunol* (2013) 173:811–8. doi:10.1111/cei.12134

81. Sleater M, Diamond AS, Gill RG. Isolelet allograft rejection by contact-dependent CD8+ T cells: perforin and FasL play alternate but obligatory roles. *Am J Transplant* (2007) 7:1927–33. doi:10.1111/j.1600-6143.2007.01889.x

82. Kim YH, Kim S, Kim KA, Yagita H, Kayagaki N, Kim KW, et al. Apoptosis of pancreatic beta-cells detected in accelerated diabetes of NOD mice: no role of Fas-Fas ligand interaction in autoimmune diabetes. *Eur J Immunol* (1999) 29:455–65. doi:10.1002/(SICI)1521-4141(199902)29:02<455::AID-IMMU455>3.0.CO;2-A

83. Thomas HE, Darwiche R, Corbett JA, Kay TW. Evidence that beta cell death in the nonobese diabetic mouse is Fas independent. *J Immunol* (1999) 163:1560–7.

84. Varanasi V, Avanesyan L, Schumann DM, Chervonsky AV. Cytotoxic mechanisms employed by mouse T cells to destroy pancreatic β-cells. *Diabetes* (2012) 61:2862–70. doi:10.2337/db11-1784

85. Rabinovitch A, Suarez-Pinzon WL. Role of cytokines in the pathogenesis of autoimmune diabetes mellitus. *Rev Endocr Metab Disord* (2003) 4:291–9. doi:10.1023/A:1025160614313

86. Dharnidharka VR, Van Patten Y, Bahjat FR, Clare-Salzler M. Fas stimulation results in selective islet infiltrate apoptosis in situ and reversal of diabetes. *Ann N Y Acad Sci* (2002) 958:160–2. doi:10.1111/j.1749-6632.2002.tb02960.x

87. Higuchi Y, Herrera P, Muniesa P, Huarte J, Belin D, Ohashi P, et al. Expression of a tumor necrosis factor alpha transgene in murine pancreatic beta cells results in severe and permanent insulinse loss without evolution towards diabetes. *J Exp Med* (1992) 176:1719–31. doi:10.1084/jem.176.6.1719

88. Picarella DE, Kratz A, Li CB, Ruddle NH, Flavell RA. 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* (1993) 150:4136–50.

89. Grewal IS, Grewal KD, Wong FS, Picarella DE, Janeway CA, Flavell RA. Local expression of transgene encoded TNF alpha in islets prevents autoimmunity diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J Exp Med* (1996) 184:1963–74. doi:10.1084/jem.184.5.1963

90. Christen U, Von Herrath MG. Apoptosis of autoreactive CD8 lymphocytes as a potential mechanism for the abrogation of type 1 diabetes by islet-specific TNF-alpha expression at a time when the autoimmune process is already ongoing. *Ann N Y Acad Sci* (2002) 958:166–9. doi:10.1111/j.1749-6632.2002.tb02962.x

91. Moritani M, Yoshimoto K, Wong SF, Tanaka C, Yamaoka T, Sano T, et al. Abrogation of autoimmune diabetes in nonobese diabetic mice and protection against effector lymphocytes by transgenic paracrine TGF-beta1. *J Clin Invest* (1998) 102:499–506. doi:10.1172/JCI2992

92. Grewal IS, Grewal KD, Wong FS, Wang H, Picarella DE, Janeway CA, et al. Expression of transgene encoded TGF-beta in islets prevents autoimmune diabetes in NOD mice by a local mechanism. *J Autoimmun* (2002) 19:9–22. doi:10.1006/jaut.2002.0599

93. Satoh J, Seino H, Abe T, Tanaka S, Shintani S, Ohta S, et al. Recombinant human tumor necrosis factor alpha suppresses autoimmune diabetes in nonobese diabetic mice. *J Clin Invest* (1989) 84:1345–8. doi:10.1172/JCI114304

94. Jacob CO, Aiso S, Michie SA, McDevitt HO, Acha-Orbea H. 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* (1990) 87:968–72. doi:10.1073/pnas.87.3.968

95. Campbell IL, Oxbrow L, Harrison LC. Reduction in insulin secretion following administration of IFN-gamma and TNF-alpha in the NOD mouse. *J Autoimmun* (1991) 4:249–62. doi:10.1016/0896-8411(91)90022-5

96. Kaminitz A, Yolcu ES, Mizrahi K, Shirwan H, Askenasy N. Killer Treg cells ameliorate inflammatory insulitis in non-obese diabetic mice through local and systemic immunomodulation. *Int Immunol* (2013) 25:485–94. doi:10.1093/intimm/dxt016

97. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* (2006) 295:2275–85. doi:10.1001/jama.295.19.2275

98. Wong AK, Kerkoutian S, Said J, Rashidi H, Pullarkat ST. Risk of lymphoma in patients receiving antitumor necrosis factor therapy: a meta-analysis of published randomized controlled studies. *Clin Rheumatol* (2012) 31:631–6. doi:10.1007/s10067-011-1895-y

99. Liu Y, Fan W, Chen H, Yu MX. Risk of breast cancer and total malignancies in rheumatoid arthritis patients undergoing TNFα antagonist therapy: a meta-analysis of randomized controlled trials. *Asian Pac J Cancer Prev* (2014) 15:3403–10. doi:10.7314/APJCP.2014.15.8.3403

100. Herrera PL, Harlan DM, Vassalli P. A mouse CD8 T cell-mediated acute autoimmune diabetes independent of the perforin and Fas cytotoxic pathways: possible role of membrane TNF. *Proc Natl Acad Sci U S A* (2000) 97:279–84. doi:10.1073/pnas.97.1.279

101. Thomas HE, Graham KL, Chee J, Thomas R, Kay TW, Krishnamurthy B. Proinflammatory cytokines contribute to development and function of regulatory T cells in type 1 diabetes. *Ann N Y Acad Sci* (2013) 1283:81–6. doi:10.1111/j.1749-6632.2012.06797.x

**Conflict of Interest Statement:** HS and EY are inventors of several patents related to Fas-ligand. NA discloses no potential conflict of interest.

Copyright © 2017 Yolcu, Shirwan and Askenasy. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.