



Invited Review

Helminth mediated modulation of Type 1 diabetes (T1D)

Paola Zaccane, Anne Cooke*

Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK

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ABSTRACT

Type 1 diabetes is increasing dramatically in incidence in the developed world. While there may be several reasons for this, improved sanitation and public health measures have altered our interactions with certain infectious agents such as helminths. There is increasing interest in the use of helminths or their products to alleviate inflammatory or allergic conditions. Using rodent models of diabetes, it has been possible to explore the therapeutic potential of both live infections as well as helminth-derived products on the development of autoimmunity. This review provides an overview of the findings from animal models and additionally explores the potential for translation to the clinic.

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1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease where the pancreatic insulin-producing beta cells are destroyed by the immune system. The development of T1D is known to be influenced by both genetic and environmental factors, as the concordance rate for identical twins is not 100% but approximately 50–60% (Redondo et al., 2001). T1D is a disease which mainly affects young people and was lethal before the discovery of insulin by Banting and Best in the 1920s (Banting and Best, 1922), which enabled restoration of glucose homeostasis. This autoimmune disease is not a new disease; the first description dates back to around 1500 BC in the Ebers Papyrus. However this disease, similar to some other inflammatory disorders such as multiple sclerosis (MS) and inflammatory bowel disease (IBD), is dramatically increasing in incidence in the developed world. As this increase in incidence is occurring faster than can be accounted for by genetic change, this suggests a change in the environment. There have been many environmental factors which have been suggested to play a role in this increased incidence of T1D including exposure to viral infection, dietary change or decreased exposure to certain infectious agents that had historically played a significant role in shaping the human immune system. The various arms of the immune system, innate and adaptive, have evolved to combat infection and the multiplicity of immune cell types and their products reflect the arms race that has occurred over many years between infectious agents and their hosts. Examination of prehistoric mummies, such as the ‘ice man’

of the Otzal Alps or a pre-Columbian mummy in Peru, have revealed the presence of mycobacteria and parasites (Aspöck et al., 1996; Gonçalves et al., 2003; Dunne and Cooke, 2005). Improved sanitation and living conditions, together with vaccination strategies, have markedly diminished our exposure to infectious agents and development of infectious disease over the last 60 years. Therefore, one relatively recent change that might play a role in the increased incidence of T1D might be the reduced exposure to infectious agents.

To test this hypothesis several studies have examined whether diabetes onset in the non-obese diabetic (NOD) murine model of T1D is influenced by infection. The NOD mouse was generated by Makino and colleagues in 1980 (Makino et al., 1980), and has provided one of most used animal models for T1D. As in human T1D, the development of diabetes is under complex genetic control with more than 40 genes implicated in susceptibility to disease onset. The pancreas of NOD mice develops mononuclear cell infiltration in the islet area of the pancreas (insulinitis) before clinical signs of disease become evident with hyperglycemia, glycosuria and substantial weight loss. T1D in NOD mice is thought to be a Th1-mediated autoimmune disease, but other immune cells are crucial for the initiation of the autoimmune process. B cells, dendritic cells (DCs) and macrophages ($M\Phi$) are found in the pancreas of pre-diabetic NOD mice, and have been implicated in autoantigen presentation and expansion of CD8+ and CD4+ autoreactive T cells and destruction of the insulin-producing β cells (Driver et al., 2011). While some infectious agents appear unable to inhibit the onset of diabetes in NOD mice (Smith et al., 2007), others such as mycobacterial or parasitic infections inhibit diabetes development (Table 1). It is difficult to pinpoint what are the exact characteristics

* Corresponding author. Tel.: +44 1223 33 3907; fax: +44 1223 33 3914.

E-mail address: ac@mole.bio.cam.ac.uk (A. Cooke).

Table 1
Bacterial and viral infections preventing or exacerbating Type 1 diabetes (T1D) in non-obese diabetic (NOD) mice. Examples of infections and infectious antigens that are shown to alter diabetes outcome in a murine model of T1D.

Infection/antigen	Species/name	Effect on diabetes	Reference
Viral infections			
	Monkey rotavirus RRV	Inhibition (at birth)	Graham et al. (2007) and Harrison (2008)
	Monkey rotavirus RRV	Acceleration (at 12 weeks)	Graham et al. (2008)
	Lymphocytic choriomeningitis virus	Prevention	Oldstone (1990) and Filippi et al. (2009)
	Lactate dehydrogenase virus	Prevention	Takei et al. (1992)
	Mouse hepatitis virus	Inhibition	Wilberz et al. (1991)
	Murine gammaherpes virus-68	Delay	Smith et al. (2007)
	Coxsackie B3 & B4 viruses	Inhibition	Tracy et al. (2002), Davydova et al. (2003) and Filippi et al. (2009)
	Coxsackie B4 virus	Acceleration (at 8 weeks)	Serreze et al. (2000)
Viral antigens			
	CpG DNA	Inhibition	Quintana et al. (2000)
	CpG DNA	No effect	Lee et al. (2005)
	Poly I:C	Prevention	Serreze et al. (1989)
Bacterial infections			
	<i>Salmonella typhimurium</i>	Prevention	Zaccane et al. (2004)
	<i>Mycobacterium avium</i>	Prevention	Bras and Aguas (1996)
Bacterial antigens			
	<i>Mycobacterium bovis</i> Bacillus Calmette-Guerin	Prevention	Harada et al. (1990) and Baxter et al. (1994)
	Complete Freund's adjuvant (<i>Mycobacteria</i>)	Prevention	Sadelain et al. (1990)
	Streptococcal OK-432	Prevention	Toyota et al. (1986)
	<i>Klebsiella pneumoniae</i> glycoprotein extract	Inhibition	Sai and Rivereau (1996)
	<i>Escherichia coli</i> LPS	Inhibition	Sai and Rivereau (1996)

RRV, rhesus monkey rotavirus; LPS, Lipopolysaccharide.

of an organism or its products that might inhibit diabetes development in NOD mice. Mouse gamma herpes virus 68 appeared an ideal candidate to inhibit diabetes development as this large DNA virus encodes a range of potent immunomodulatory components yet it is unable to provide long-term inhibition of diabetes development irrespective of time of infection (Smith et al., 2007). Other virus infections, such as that by group B coxsackieviruses, prevent diabetes onset if young NOD mice are infected but may accelerate onset in older mice, in the latter case through replication in the pancreatic β cell (Tracy et al., 2010). Infection of NOD mice with an attenuated *Salmonella typhimurium* optimally prevents diabetes onset if infection occurs at approximately 8 weeks of age while helminth infections optimally inhibit if infection occurs at approximately 4 weeks of age (Cooke et al., 1999; Zaccane et al., 2004). The mechanisms implicated in diabetes prevention may vary from organism to organism.

Initial studies showed that infection with *Schistosoma mansoni* could prevent diabetes onset in NOD mice. Subsequent studies con-

firmed the potent effect of other helminth infections on T1D in NOD mice including *Trichinella spiralis*, *Heligmosomoides polygyrus*, *Litomosoides sigmodontis* and *Dirofilaria immitis* (Table 2). Protection from diabetes was associated with different patterns of response induced by the differing infectious agents. These included an increased Th2 response, increased generation of regulatory T (Treg) cells, production of IL-10 by T and B cells and reduction in infiltrating CD8⁺T cells in the pancreas; all of which would have the potential to inhibit diabetes onset. In many cases of diabetes prevention following helminth infection the defining mechanisms have not been fully clarified. However, in the case of infection with *L. sigmodontis*, transforming growth factor (TGF) β production was found to be indispensable for diabetes prevention whereas a Th2 switch or Treg development appeared redundant (Hubner et al., 2009). The use of defined parasite products to influence diabetes onset provides an additional way of addressing mechanisms of diabetes prevention.

In the context of diabetes the observation that parasite infections might be able to influence metabolism through effects

Table 2
Helminth infection and helminth antigen preventing Type 1 diabetes (T1D). Examples of diabetes prevention in animal models of T1D using helminth infections and helminth-derived antigens.

Helminth infections	Infection	Effect	Putative mechanism	References
Helminth antigens	<i>Schistosoma mansoni</i>	Inhibition	Shift to Th2-type response	Cooke et al. (1999)
	<i>Trichinella spiralis</i>	Prevention	Shift to Th2-type response	Saunders et al. (2007)
	<i>Heligmosomoides polygyrus</i>	Prevention	Shift to Th2-type response	Saunders et al. (2007) and Liu et al. (2009)
	<i>Litomosoides sigmodontis</i>	Prevention	Shift to Th2-type response and Treg increase	Hubner et al. (2009)
	<i>L. sigmodontis</i> antigen	Inhibition	Shift to Th2-type response and Treg increase	Hubner et al. (2009)
	<i>Dirofilaria immitis</i> IgE-inducing antigen	Prevention	Shift to Th2-type response	Imai et al. (2001)
	<i>S. mansoni</i> eggs	Prevention	Shift to Th2-type response	Cooke et al. (1999) and Zaccane et al. (2003)
	<i>S. mansoni</i> soluble worm antigen (SWA)	Prevention	Expansion of iNKT cells, Shift to Th2-type response	Zaccane et al. (2003)
	<i>S. mansoni</i> soluble egg antigen (SEA)	Prevention	Expansion of alternatively activated macrophages, tolerogenic DCs, iNKT cells, Shift to Th2-type response and induction of Treg	Zaccane et al. (2003)
	<i>S. mansoni</i> LNFPIII	Prevention	Shift to Th2-type response	Atochina and Harn (2006) and Harn et al. (2009)

Treg, regulatory T cells; iNKT, invariant natural killer T; DC, dendritic cells; LNFPIII, lacto-N-fucopentaose III.

on adipose tissue is of particular interest. The presence of M1 MΦs in adipose tissue is associated with obesity, insulin resistance and impaired glucose tolerance whereas an M2 anti-inflammatory MΦ phenotype is found in lean animals with normoglycaemia. The work of Locksley and colleagues has suggested an additional route by which parasite infections or their products might influence diabetes, insulin resistance and glucose homeostasis (Wu et al., 2011). In these studies, *Nippostrongylus brasiliensis* infection was associated with an accumulation of eosinophils in adipose tissue, leading to a predominant M2 MΦ phenotype resulting in an improved ability of the infected obese mice to maintain glucose homeostasis.

2. Involvement of Tregs and natural killer T (NKT) cells in helminth mediated diabetes prevention

2.1. Tregs and NKT cells in T1D

One hypothesis regarding the pathogenic mechanisms leading to T1D suggests that impairment of immunoregulatory mechanisms might result in disproportional activity of diabetogenic T cells, with consequential breakdown of peripheral tolerance and development of autoimmunity. Specifically, two immune cell types have been discussed in the context of tolerance breakdown in T1D: Tregs (Josefowicz et al., 2012) and invariant chain natural killer T (NKT) cells (Bendelac et al., 2007).

Since the discovery that Tregs are fundamental in maintaining self-tolerance (Hori et al., 2003; Wing and Sakaguchi, 2010) the challenge has been to address their role in T1D. Some reports describe a correlation between diabetes and defects in Treg number or function (Brusko et al., 2005; Lindley et al., 2005; Szypowska et al., 2012), while others show no difference in Treg number and distribution between T1D patients and healthy controls and, rather, suggest that the effector cells have become refractory to immune regulation (Brusko et al., 2007; D'Alise et al., 2008; Long et al., 2010). Although the role of Treg impairment in the pathogenesis of T1D still needs to be clarified, there is increasing evidence to support the importance of Treg expansion in controlling autoimmunity (Daniel and von Boehmer, 2011; Leslie, 2011).

Similar to Tregs, the study of NKT cells in the initiation/progression of T1D has produced contradictory results (Wilson et al., 1998; Kukreja et al., 2002; Lee et al., 2002; Oikawa et al., 2002; Michalek et al., 2006; Tsutsumi et al., 2006; Kis et al., 2007; Montoya et al., 2007; Roman-Gonzalez et al., 2009; Berzins et al., 2011). However, their ability to promote immunomodulation for the treatment of diabetes has been demonstrated in animal models of T1D (Fletcher and Baxter, 2009) and their potential to prevent autoimmune diseases such as diabetes represents an important clinical target (Novak and Novakova, 2012).

2.2. Helminths and T1D; it is not only Th2

Early work to demonstrate that helminth infections can prevent T1D was based on the premise that the Th2-immune bias (induced by the infection) would control Th1-mediated autoimmune diseases such as diabetes (Table 2). Immunity to helminths is generally regarded as a Th2 response, but in fact numerous cell types are involved in the initiation, amplification, control and maintenance of “Type-2 immunity” (Allen and Maizels, 2012), and both Tregs and NKT cells are found to be key players in orchestrating a balanced immune response to helminth infections. The expansion of Tregs, reported in different helminth infections, has the essential role of controlling excessive inflammation, dampening the Th2 response and containing tissue damage (Taylor et al., 2012). Less understood (but not less relevant) is the function of NKT cells in the modulation of the host immune response in

helminthic infection (Tupin et al., 2007; Faveeuw et al., 2008). Invariant NKT (iNKT) and non-invariant NKT (non-iNKT) cells have a major impact on the immune response to helminths and, as an early source of powerful polarizing cytokines such as IFN- γ and IL-4, these cells play key roles in influencing the Th1 or Th2 balance of responses (Mallevaey et al., 2007).

In recent years, data generated from the NOD mouse model of T1D (Driver et al., 2011) has added complexity to the simple idea that a Th2-shift can prevent Th1-autoimmunity, moving the focus of attention to innate and regulatory cell populations. For example, helminth infection studies in IL-4-deficient NOD mice suggest that other regulatory cytokines (IL-10 and TGF- β) are responsible for diabetes prevention (Hubner et al., 2012; Mishra et al., 2012).

2.3. NKT cells, helminths and T1D

Helminth extracts or purified products have been used in both in vivo and in vitro studies to further clarify the mechanisms by which helminths modulate inflammatory responses such as T1D (Table 2). For example, soluble extracts of both the worm (SWA) and the eggs (SEA) of *S. mansoni* confer diabetes prevention when administered to 4–6 week old NOD mice (Zaccane et al., 2003). Detailed cellular analyses and cell transfer studies have shown that these two different extracts mediate diabetes prevention through distinct mechanisms. SEA treatment of female NOD mice is associated with induction of Tregs, in addition to a Th2 response and B and T cell IL-10 production, and transfer studies have highlighted the critical role the Tregs play in modulating diabetes onset (Zaccane et al., 2003, 2009). These cells were not found to play such a key role in SWA-mediated diabetes prevention but iNKT cells induced by these helminth antigens have been proposed to play a prominent role (Zaccane et al., 2003). Underlying these effects on Tregs are helminth product-induced phenotypic and functional changes in DC and MΦ populations (Allen and Maizels, 2012). Glycoconjugate antigens (including glycolipids) produced by helminths are detected by pattern recognition receptors (PRRs) on many cell types (van Die and Cummings, 2010). A key role for NKT cells in diabetes modulation has been highlighted in animal models where iNKT deficiency in the NOD mouse has been linked to diabetes (Poulton et al., 2001) and the activation/expansion of iNKT cells using glycolipids can correct this deficiency and prevent diabetes (Hong et al., 2001; Sharif et al., 2001).

A role for non-iNKT cells in T1D has also been demonstrated in co-transfer experiments using NKT cells from transgenic NOD mice overexpressing a CD1d-restricted T cell receptor (TCR; V α 3.2 + V β 9+) to control diabetogenic BDC 2.5 CD4+ T cells (Kadri et al., 2012). This study demonstrates that non-iNKT cells are able to regulate diabetogenic CD4+ T cells without the cooperation of other T cell types and that the PD-1/PD-L1 pathway is an essential component of the mechanism. Interestingly, the PD1/PD-L1 pathway is a key regulator in T1D (Sharpe et al., 2007; Francisco et al., 2010), and is also a pathway activated in helminth infections (and by helminth antigens) to inhibit T cell immunity (Smith et al., 2004; Huber et al., 2010; Hams et al., 2011). However, although iNKT cells are expanded in NOD mice following SWA treatment, the exact mechanism by which they mediate diabetes prevention remains to be clarified.

2.4. Crosstalk between NKT, DC and Treg cells

The potential involvement of helminth antigen-activated NKT cells in T1D prevention might arise from multiple mechanisms, including: direct interactions with diabetogenic cells, cytokine secretion, amplification of Th2 responses, recruitment of tolerogenic DCs, and cooperation with other immune cells (Fig. 1). In NOD mice treated with the synthetic glycolipid, α -galactosylceramide

(α -Gal-Cer), iNKT cells polarized a Th2 shift towards IL-4 and mice were protected from diabetes, while IL-4-deficient NOD mice were not, demonstrating the ability of iNKT cells to drive an anti-diabetogenic Th2 switch (Mi et al., 2004). The interplay between DC and iNKT cells is also a possible mechanism of diabetes protection: in NOD mice, treatment with α -Gal-Cer induces recruitment of tolerogenic DCs to the pancreatic lymph nodes (PLNs), where they subsequently tolerize diabetogenic T cells (Naumov et al., 2001; Chen et al., 2005). Helminth antigens have been shown to modify DCs such that they polarise to a Th2 response (MacDonald et al., 2002) and also convert DCs to what has been termed tolerogenic DCs that initiate Treg responses (Liu et al., 2011; Smith et al., 2011). There is a link between activated iNKT and Treg cells in diabetes prevention, since Treg cell depletion in NOD mice treated with α -Gal-Cer results in a high incidence of T1D (Ly et al., 2006).

The cross-talk between DC, NKT and Treg cells is an essential common pathway in the modulation of both T1D and immunity to helminths, making helminth-derived molecules excellent tools in the study of diabetes regulation (Fig. 1). The SEA from *S. mansoni* can prevent diabetes in NOD mice, inducing tolerogenic phenotypic changes in DCs that can expand not only Th2 cells, but also CD4⁺ Foxp3⁺ Treg cells in the pancreas of NOD mice (Zaccane et al., 2003, 2009). Phenotypic changes induced by SEA in antigen presenting cells include upregulation of cytokines (IL-2, IL-10 and TGF- β) and molecules (integrin β -8, DEC-205, Galectin-3) known to support the expansion of CD4⁺ Foxp3⁺ Tregs and downmodulate Th1 responses. Furthermore, SEA induces alternative activation in M Φ (aaM Φ) (Zaccane et al., 2010), which also are able to contain Th2 responses, inhibit Th1 and support CD4⁺ Foxp3⁺ Treg expansion (Barron and Wynn, 2011; Broadhurst et al., 2012). It is

interesting to note that SEA injection in NOD-SCID mice (which lack both mature B and T cells, as well as NKT cells) only induces incomplete alternative activation of M Φ , suggesting a reinforcing loop between the adaptive and innate arms of the immune response for alternative activation of M Φ (Burton and Zaccane, unpublished observation). While not linked to diabetes protection, the expansion of CD4⁺ Foxp3⁺ Tregs and aaM Φ is also seen in the PLN of *H. polygyrus*-infected NOD mice (Liu et al., 2009). The mechanism/s of diabetes prevention by *H. polygyrus* have been mainly attributed to Th2-immunity in intact NOD mice (Saunders et al., 2007), and described as independent of CD25 and IL-10 expression (Liu et al., 2009). However, recent work in IL-4-deficient NOD mice infected with *H. polygyrus* has revealed that IL-10 is required to mediate diabetes protection in the absence of IL-4 (Mishra et al., 2012). Furthermore, the fact that *H. polygyrus* can prevent diabetes onset even when given relatively late in the evolution of this autoimmune disease when NOD mice are 12 weeks of age suggests that mechanisms other than Th2 skewing are of importance.

Together with the expression of Foxp3 in T cells, IL-10 plays an important role in the regulation of both infections and autoimmune diseases (Ouyang et al., 2011). IL-10 modulates the function of antigen-presenting cells by inhibiting the expression of Major Histocompatibility Complex (MHC) and costimulatory molecules, which in turn induces immune suppression or tolerance. Additionally, IL-10 downregulates the expression of Th1 cytokines and reinforces T-regulatory responses. IL-10 producing T cells (Tr1) are generated during parasitic infections and play a major role in controlling the host effector response (Mills and McGuirk, 2004). Besides Th2 and CD4⁺ Foxp3⁺ T cells, Tr1 cells are also found in the pancreas of NOD mice treated with SEA (Zaccane et al., 2010).

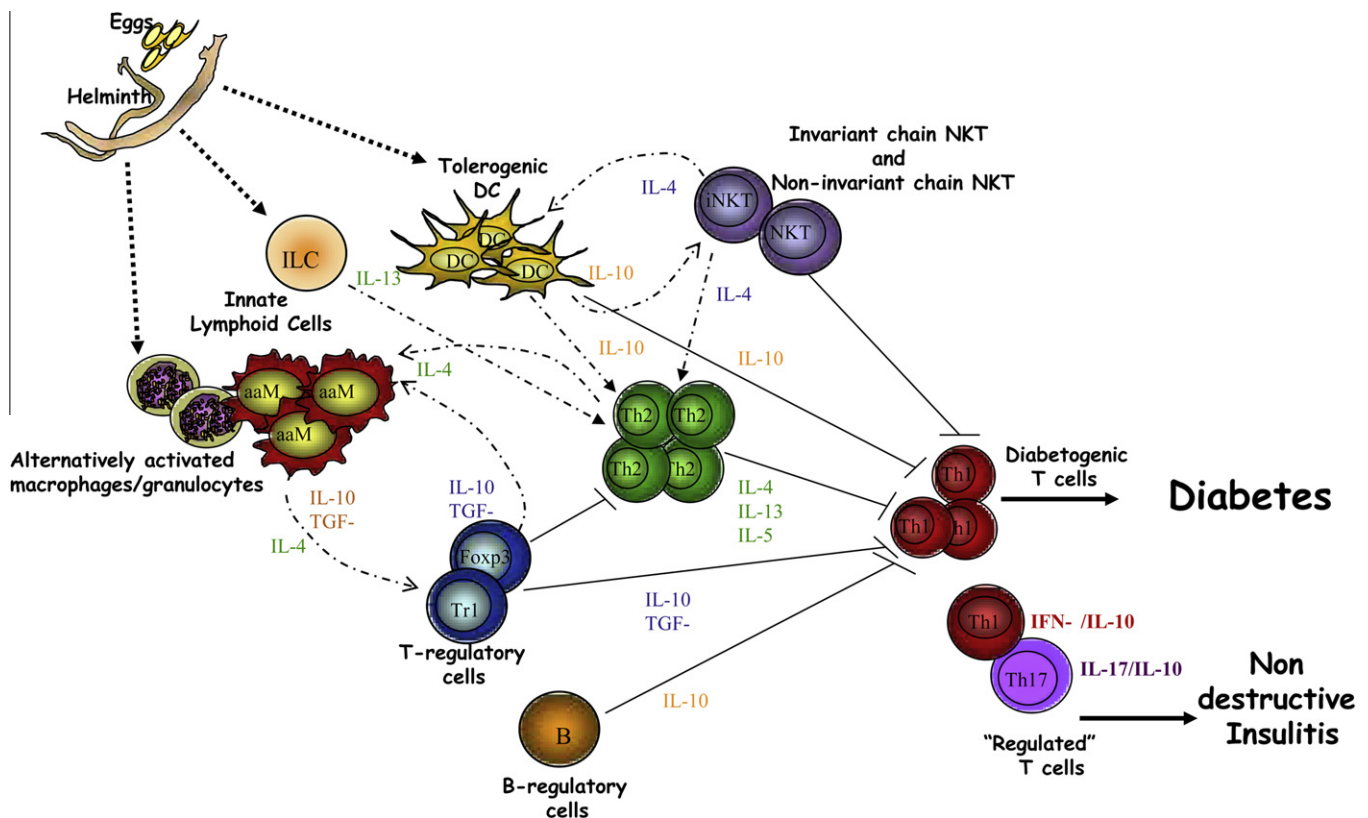


Fig. 1. Proposed model for the involvement of regulatory T cells (Tregs) and natural killer T (NKT) cells in helminth antigen-mediated diabetes protection. In the context of type 1 diabetes (T1D) prevention, Type-2 immunity to helminth antigens involves several classes of immune cells: B cells, tolerogenic dendritic cells (DCs), alternatively activated macrophages (M Φ)/granulocytes and innate lymphoid cells (ILCs) provide the right cytokine environment to induce Th2, NKT and Treg generation/expansion. In turn, cytokine secretion from NKT, Th2 and Tregs feeds back and maintains the tolerogenic and alternatively activated phenotypes of antigen presenting cells. As a result, diabetogenic (Th1) cells become regulated, inducing only a non-destructive insulinitis.

The Tr1, IL-10/IFN- γ (and IL-10/IL-17, Burton and Zaccane, unpublished data) double-expressing CD4⁺ T cells might also participate in diabetes prevention by *S. mansoni* SEA (Pot et al., 2011).

Induction of tolerance by helminth infections and products is complex and can be accomplished even when key regulatory pathways are absent or blocked. Infection with *L. sigmodontis* prevents diabetes in NOD mice and protection is associated with a Th2 shift and an increase in CD4⁺ CD25⁺ Foxp3⁺ Treg cells (Hubner et al., 2009). Recent work from the same group has established that protection could also be achieved in the absence of a Th2 immune shift and, in adoptive transfer experiments, further demonstrated that depletion of Foxp3⁺ cells from splenocytes of *L. sigmodontis*-infected mice did not increase diabetes incidence (Hubner et al., 2012). These intriguing results suggest that helminth-induced protection from diabetes is probably not dependent on continuous immune modulation by Foxp3⁺ regulatory T cells and, when a new equilibrium between immune cells has formed, the established tolerance is stable. Treg and iNKT cells play a critical role in the development and resolution of both infections and autoimmune diseases and, for this reason, represent attractive targets for the development of immunotherapies. In addition to NKT and Treg cells, the newly identified family of innate lymphoid cells (ILCs) (Spits and Di Santo, 2012), which also are involved in parasitic and inflammatory diseases, should be evaluated as potential players in diabetes prevention and as potential targets for helminth immunotherapy. Another under-investigated population of cells which may play a role in helminth infections is $\gamma\delta$ T cells (Inagaki-Ohara et al., 2011). In seeking ways to enhance cellular regulation to induce tolerance in inflammatory/autoimmune disease, making use of helminth-derived products is an interesting prospect, but is not without its challenges. Suitable and safe helminth-derived molecules will require detailed characterisation before finding applications in clinical trials.

3. Use of parasite antigens to define mechanisms of protection

While much has been done using helminth extracts such as SEA and SWA in diabetes studies and, indeed, in studies of other autoimmune conditions, relatively little work has been done with purified helminth antigens. ES-62, the excretory/secretory purified product derived from *Acanthocheilonema viteae*, has been shown to have powerful immunodulatory effects on collagen-induced arthritis and experimental allergic encephalomyelitis, animal models of rheumatoid arthritis and multiple sclerosis, respectively. However, ES-62 has no effect on development of diabetes in NOD mice (Harnett and Cooke, unpublished observations). This may reflect the fact ES-62 has an effect on Th17 responses, with little evidence of an effect on Th1-mediated diseases (Pineda et al., 2012). In contrast, the excretory/secretory component of *D. immitis*, which had been previously shown to induce non-specific IgE, was able to inhibit diabetes development in NOD mice when administered in a recombinant form, rDiAg, to 6 week old NOD mice (Imai et al., 2001). The presumed mechanism of action of rDiAg is through induction of a dominant islet-specific Th2 response.

Although defined products of *S. mansoni* have not thus far been tested for their capacity to inhibit T1D development in NOD mice, possible mechanisms by which major components of SEA such as IPSE/ α 1 and omega-1 might influence diabetes have been assessed through in vitro and in vivo studies. These studies have highlighted the ability of omega-1 to be a major Th2 inducing component in SEA (Everts et al., 2009; Steinfelder et al., 2009). IPSE/ α 1 has been shown to induce IL-4 from basophils and skew T cell immune responses towards Th2 (Schramm et al., 2007).

Recent studies are shedding light on the receptors that are engaged by defined helminth antigens in their interactions with

DCs. Earlier studies using helminth extracts such as SEA had shown involvement of C-type leptin receptors (CLRs), such as DC-SIGN, mannose receptor and M Φ galactose type lectin, in DCs where engagement resulted in internalisation of SEA and translocation to MHC class II-containing lysosomal compartments (van Liempt et al., 2007). Other studies had shown interactions of SEA with the CLR, dectin-2, resulting in activation of the inflammasome and IL-1 β production (Ritter et al., 2010). Omega-1 has been shown to be internalised by the mannose receptor and then, via its RNase activity, to degrade RNA and impair protein synthesis (Everts et al., 2012). Studies in NOD mice have shown that SEA expands Foxp3 expressing Treg cells in the pancreas of NOD mice and induces these cells from naïve T cells in vitro in co-cultures with DCs (Zaccane et al., 2009). The data obtained in NOD mice showed that induction of Foxp3 expression in CD4⁺ T cells by SEA was due to the effects of this helminth extract not only on DCs, but also directly on T cells (Zaccane et al., 2009; Burton et al., 2010). In these studies DEC-205 and TLR2 were shown to be involved. Similar to SEA, omega-1 (but not IPSE/ α 1), was also shown to expand Foxp3 expressing T cells in vivo and in DC/T cell co-cultures in vitro, inducing both TGF- β and the retinoic acid (RA) converting enzyme, retinaldehyde dehydrogenase 2 (Raldh2), in DCs (Zaccane et al., 2011).

Interestingly, increased production of IL-1 β was observed in lipopolysaccharide (LPS) and omega-1-stimulated NOD DCs, paralleling the observations made with SEA by Ritter and colleagues (Ritter et al., 2010). Upregulation of Raldh2 in aaM Φ has recently been shown to be induced by *S. mansoni* infection (Broadhurst et al., 2012). These aaM Φ are able to induce Foxp3 expression in T cells, a process which is much increased in the presence of TGF- β and is dependent on RA. The induction of Th2 responses, coupled with the capacity to induce Tregs in the case of omega-1, would suggest that agents such as IPSE and omega-1 would be able to inhibit diabetes. It is possible that these agents might be more effective if given in combination, either together or with an islet antigen such as insulin.

4. Are there any possible negative consequences of using helminth-derived products?

Chronic infection with helminths is characterised by Type-2 immunity and downregulation of the host immune response. Interestingly, Th2 immunity to helminth infections is not associated with allergy; in fact, despite high levels of IgE, granulocyte release of antimicrobial-inflammatory molecules is restrained by multiple regulatory mechanisms, in particular by IL-10 (Yazdanbakhsh et al., 2002). Suppression of the host immune system could present problems such as reduced immune response to infection (Semenya et al., 2012) and decreased efficacy of vaccination (Elias et al., 2005). Immuno-suppression might also result in impaired anti-tumour immunity, due to expansion of Tregs and aaM Φ , and upregulation of IL-10, TGF- β and CTLA-4 (Baecher-Allan and Anderson, 2006; Beyer and Schultze, 2006; Sica and Bronte, 2007). However these effects, which have been associated with live helminth infections, may not be observed when defined helminth products are employed. In the case of a live infection, a whole range of immune modulators may be produced which are able to elicit different immunological responses, while immunization with a defined helminth-derived product might not induce the same “balanced” immuno-regulation that the complete range of helminth antigens might induce in the host. Further, in the case of schistosomiasis, a live infection might cause tissue damage (inflicted by the presence of the parasite), which would not be induced by exposure only to defined products. As an additional consideration, certain helminth infections have been associated with increased incidence

of multiple cancers, including cervical, colorectal and bladder, among others (Bouvard et al., 2009). Although these observations have been in the context of live helminth infection, evidence exists that antigens from *Schistosoma haematobium* may also have tumour promoting effects and, thus, the carcinogenic capacity of other helminth antigens should also be investigated (Botelho et al., 2009). The study of appropriate helminth-derived products to induce tolerance will have to consider all of the possible side effects that dysregulated Type-2 immunity and immune suppression might cause.

5. What hurdles need to be overcome for transfer to the clinic?

T1D is classically a disease of juvenile onset and individuals with this disease currently survive through administration of exogenous insulin. Any helminth-based therapy which is introduced would need to be used on young children and current data suggests that therapy would have to be introduced before diabetes has commenced. As the concordance rate for diabetes development in identical twins is approximately 50–60%, identifying susceptible individuals as they enter a pre-diabetic phase is not straightforward in the absence of robust biomarkers. Ideally, highly purified products of helminths would be employed and there would need to be certainty that such products would themselves not have untoward side effects or render the recipient more susceptible to other diseases. Immunogenicity of parasite-derived products may impede long-term use. More research into the mechanisms by which helminth infections and their products modulate the onset of autoimmune pathology, together with identification of the cell types and pathways employed in interactions with helminth-derived products, will enable the identification of new targets and the development of small molecules to affect their function.

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