

REVIEW

IAPP and type 1 diabetes: implications for immunity, metabolism and islet transplants

Heather C Denroche¹ and C Bruce Verchere^{1,2}¹Department of Surgery, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada²Department of Pathology and Laboratory Medicine, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, CanadaCorrespondence should be addressed to C B Verchere: bverchere@bcchr.ca

Abstract

Islet amyloid polypeptide (IAPP), the main component of islet amyloid in type 2 diabetes and islet transplants, is now recognized as a contributor to beta cell dysfunction. Increasingly, evidence warrants its investigation in type 1 diabetes owing to both its immunomodulatory and metabolic actions. Autoreactive T cells to IAPP-derived epitopes have been described in humans, suggesting that IAPP is an islet autoantigen in type 1 diabetes. In addition, although aggregates of IAPP have not been implicated in type 1 diabetes, they are potent pro-inflammatory stimuli to innate immune cells, and thus, could influence autoimmunity. IAPP aggregates also occur rapidly in transplanted islets and likely contribute to islet transplant failure in type 1 diabetes through sterile inflammation. In addition, since type 1 diabetes is a disease of both insulin and IAPP deficiency, clinical trials have examined the potential benefits of IAPP replacement in type 1 diabetes with the injectable IAPP analogue, pramlintide. Pramlintide limits postprandial hyperglycemia by delaying gastric emptying and suppressing hyperglucagonemia, underlining the possible role of IAPP in postprandial glucose metabolism. Here, we review IAPP in the context of type 1 diabetes: from its potential involvement in type 1 diabetes pathogenesis, through its role in glucose metabolism and use of IAPP analogues as therapeutics, to its potential role in clinical islet transplant failure and considerations in this regard for future beta cell replacement strategies.

Key Words

- ▶ amylin
- ▶ islet amyloid polypeptide
- ▶ autoimmune
- ▶ inflammation
- ▶ pancreatic beta cell

*Journal of Molecular
Endocrinology*
(2018) **60**, R57–R75

Introduction

Islet amyloid polypeptide (IAPP or amylin), the main constituent of insoluble amyloid deposits in pancreatic islets of individuals with type 2 diabetes (T2D), is secreted from pancreatic beta cells at a level of approximately 1% that of insulin (Kautzky-Willer *et al.* 1994, Dechenes *et al.* 1998, Kahn *et al.* 1998, Knowles *et al.* 2002). The unique property of IAPP to aggregate and form islet amyloid is implicated in the pathology of T2D due to its toxic effects on beta cells and, more recently, its pro-inflammatory properties. Islet amyloid is present in the majority of

persons with T2D and absent in most persons without T2D (Hull *et al.* 2004). Unlike human IAPP (hIAPP), rodent IAPP (rIAPP) does not aggregate, due to the presence of three proline residues within the amyloidogenic region of the peptide; rodents expressing transgenic hIAPP in beta cells develop extensive islet amyloid on high-fat diet, accompanied by beta cell dysfunction and loss. Why IAPP aggregates form in T2D, and their precise role in disease pathogenesis remains unclear. In its monomeric form, IAPP may help regulate metabolism and satiety, though

this biological role remains a matter of some debate. These properties of monomeric IAPP have been exploited for the treatment of both type 1 diabetes (T1D) and T2D, as well as obesity.

T1D, representing approximately 10% of diabetes cases and increasing in prevalence, results from the autoimmune destruction of beta cells. While islet amyloid has long been studied for its role in T2D pathogenesis, little is known about its role, if any, in T1D. IAPP has been reported to be an autoantigen in both T1D and the non-obese diabetic (NOD) mouse model of spontaneous autoimmune diabetes (Panagiotopoulos *et al.* 2003, Ouyang *et al.* 2006, Standifer *et al.* 2006, Delong *et al.* 2011, 2016, Baker *et al.* 2013, Wiles *et al.* 2017). In addition, recent evidence has established IAPP aggregates as strong proinflammatory stimuli that elicit interleukin-1B (IL1B) secretion from innate immune cells (Masters *et al.* 2010, Westwell-Roper *et al.* 2011, Sheedy *et al.* 2013). Intriguingly, islet amyloid forms rapidly in islets transplanted into T1D recipients and likely plays a role in islet graft inflammation and dysfunction (Andersson *et al.* 2008, Udayasankar *et al.* 2009, Potter *et al.* 2010, 2015, Westwell-Roper *et al.* 2011, Westermark *et al.* 2012). In this review, we examine the underexplored yet plausible role for IAPP in T1D pathophysiology, the use of IAPP analogues for T1D treatment, and islet amyloid as a potential contributor to clinical islet transplant failure.

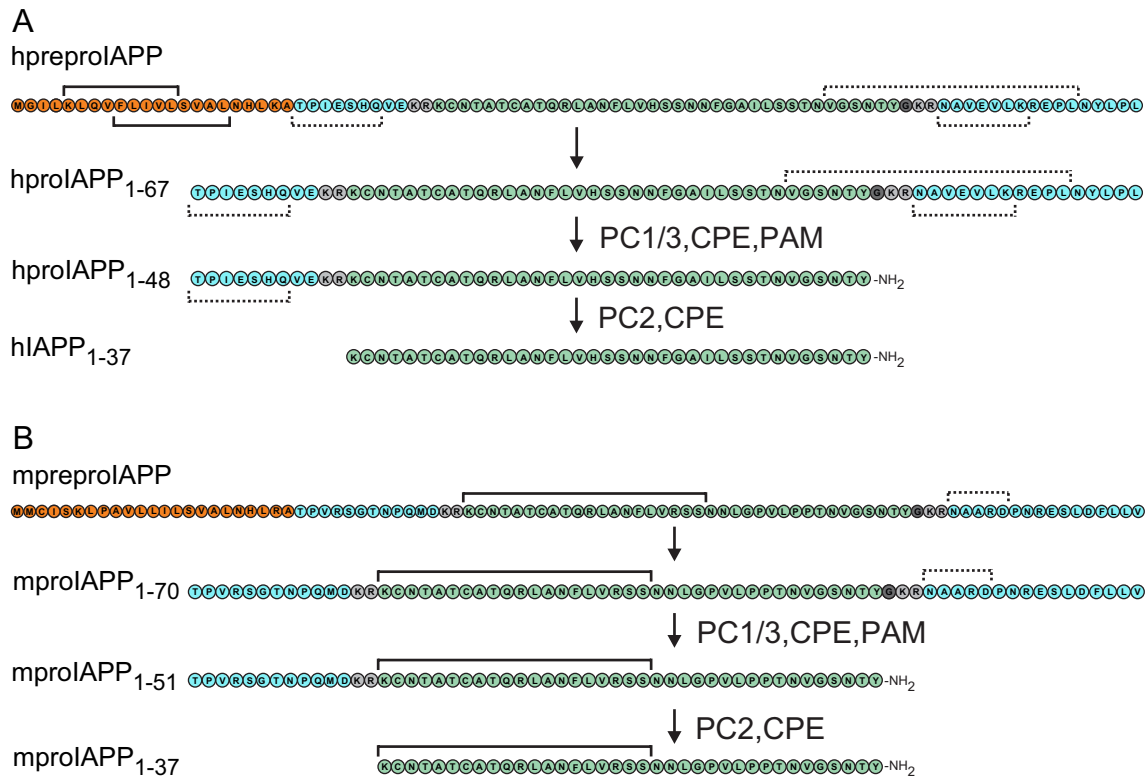
IAPP as an autoantigen in type 1 diabetes

Inappropriate targeting of self-peptides (autoantigens) by the adaptive immune system is central to autoimmune diseases. In T1D, autoimmune recognition of beta cell antigens leads to the progressive destruction of beta cells. A similar process occurs spontaneously in the NOD mouse, leading to extensive beta cell loss and diabetes, though on a shorter time scale than in human T1D; this model, sharing many similar features with human T1D, has been used extensively to research the immunological processes underlying T1D (Thayer *et al.* 2010). Many beta cell antigens, targeted by one or both of the humoral (autoantibodies) and cellular (autoreactive T cells) branches of adaptive immunity, have been identified in NOD mice and humans (Roep & Peakman 2012). Islet autoantibodies are strongly associated with T1D, with insulin, glutamic acid decarboxylase 2 (GAD2, also known as GAD65) and protein tyrosine phosphatase, receptor type N (PTPRN, also known as IA2) autoantibodies often appearing well before diabetes onset. Though circulating islet autoantibodies are predictive of T1D, there is little

evidence for their role in disease progression. In contrast, autoreactive T cells are considered causal in beta cell killing and disease progression, and numerous T cell-targeted beta cell autoantigens have been identified in NOD mice and confirmed in humans (Panagiotopoulos *et al.* 2004). T cells autoreactive to insulin and its precursor forms are prevalent in both humans with T1D and NOD mice. Most autoreactive T cell targets are beta cell specific, including glucose-6-phosphatase catalytic subunit 2 (G6PC2, also known as IGRP) and solute carrier family 30 member 8 (SLC30A8 also known as ZNT8); others are not entirely restricted to beta cells, including chromogranin A and PTPRN. Studies in NOD mice have shed some light on the relative roles of individual autoantigens: deletion of either proinsulin gene alters diabetes progression in NOD mice (Moriyama *et al.* 2003, Thébault-Baumont *et al.* 2003, Nakayama *et al.* 2007), whereas deletion of GAD or PTPRN does not alter disease progression (Yamamoto *et al.* 2004, Kubosaki *et al.* 2005).

Evidence suggesting IAPP is a prevalent autoantigen in diabetes has emerged. IAPP and its precursor forms contain several epitopes that have been found to activate either CD4 or CD8 T cells in NOD mice and T1D subjects (Panagiotopoulos *et al.* 2003, Ouyang *et al.* 2006, Standifer *et al.* 2006, Delong *et al.* 2011, 2016, Baker *et al.* 2013, Wiles *et al.* 2017). IAPP autoantibodies have also been identified in humans, but do not associate specifically with T1D, precluding a causal role in diabetes (Clark *et al.* 1991, Gorus *et al.* 1992). Though IAPP-reactive T cells associate with T1D, much remains to be gleaned concerning their role in diabetes, particularly in light of the numerous beta cell autoantigens identified to date and those yet to be identified. Notably, as with GAD2 and PTPRN, genetic deletion of IAPP does not alter diabetes progression in NOD mice, indicating IAPP is not an essential islet autoantigen in this model (Baker *et al.* 2013).

To delineate the distinct autoimmune epitopes within IAPP and its precursor forms, a brief description of proIAPP processing in beta cells is necessary. Like insulin, IAPP is initially translated as an immature prohormone precursor (preproIAPP) (Fig. 1); removal of the signal peptide yields proIAPP (in humans: hproIAPP₁₋₆₇; in mice: mproIAPP₁₋₇₀). The proIAPP processing pathway has been largely elucidated from studies in mice and is assumed, but has not been shown, to be similar in humans. ProIAPP is first cleaved by prohormone convertase (PC) 1/3 to release a C terminal fragment and, following carboxypeptidase E (CPE)-mediated removal of dibasic residues, an N-terminally extended intermediate (in humans hproIAPP₁₋₄₈; in mice mproIAPP₁₋₅₀).

**Figure 1**

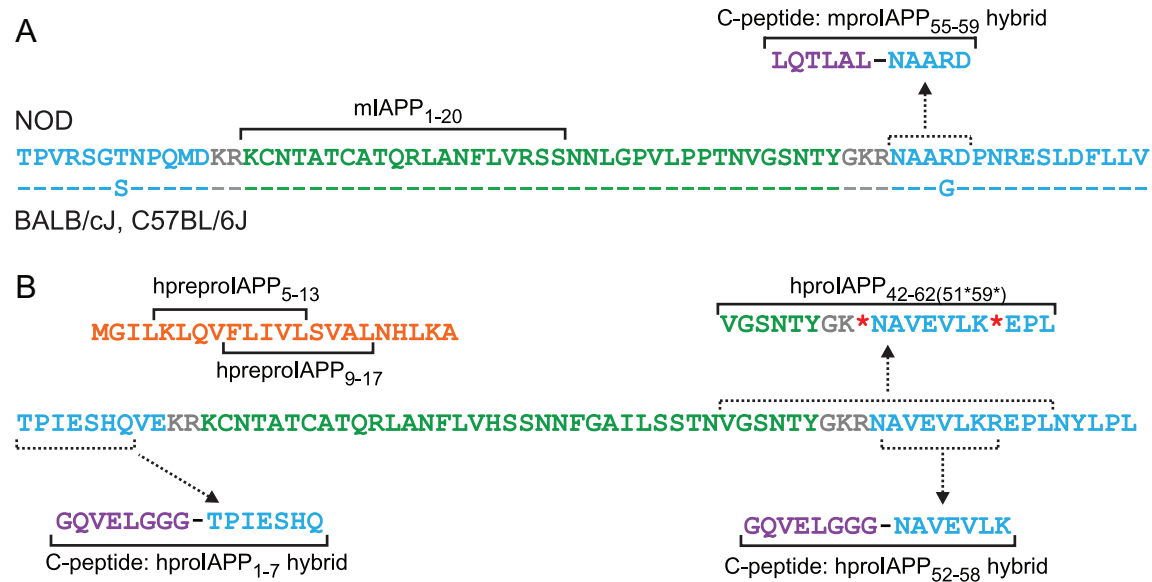
ProlIAPP processing in humans and mice. Schematic of proIAPP processing in humans (A) and mice (B). The initially translated IAPP prepropeptide contains a signal peptide (orange residues) linked to a proIAPP precursor containing the final IAPP peptide (green residues), flanked by N and C terminal flanking regions (blue residues); the N and C terminal flanking regions are also termed IAPP1 and IAPP2, respectively, by some groups. Epitopes are indicated in solid lines. Broken lines indicate residues within the peptide that form epitopes following additional modifications. The signal peptide is cleaved from preproIAPP to produce the proIAPP peptide (hproIAPP₁₋₆₇ in humans; mproIAPP₁₋₇₀ in mice). The proIAPP precursor is subsequently cleaved by PC1/3 to release the C-terminal-flanking region and an N-terminally extended proIAPP intermediate (hproIAPP₁₋₄₈ in humans; mproIAPP₁₋₅₁ in mice). These are likely cleaved by PC2 to release the N-terminal-flanking region and mature IAPP (hIAPP₁₋₃₇ in humans; mIAPP₁₋₃₇ in mice). CPE removes the basic residues (grey) after each PC cleavage. The glycine residue (dark grey) is removed and C-terminal tyrosine amidated by the enzyme peptidylglycine alpha-amidating monooxygenase (PAM).

This proIAPP intermediate is then cleaved by PC2 to release an N-terminal fragment and the final 37-amino acid hIAPP peptide (hIAPP₁₋₃₇ or mIAPP₁₋₃₇). Epitopes have been identified by ourselves and other groups in the signal peptide of preproIAPP, in the N- and C-terminal regions of proIAPP, as well as in the mature form of IAPP. There is some variability in the nomenclature of these epitopes in the literature; in this review, we refer to them based on their amino acid positions in their respective IAPP precursor or mature forms.

IAPP epitopes targeted by CD4 T cells

The Haskins group originally described a CD4 T cell clone isolated from NOD mice (BDC 6.9) that responded to an autoantigen present in islets from NOD but not BALB/c mice (Dallas-Pedretti *et al.* 1995). This unknown islet autoantigen was linked to a region on chromosome

6 near the *Iapp* locus. Subsequent analyses revealed polymorphisms in the NOD *Iapp* sequence conferring two single amino acid substitutions in the proIAPP peptide (Fig. 2A). However, a number of synthetic peptides derived from NOD mproIAPP were unable to directly stimulate BDC 6.9 cells, and the precise epitope remained elusive for two more decades. Through screening with a fusion peptide library, the Haskins group recently discovered that the epitope recognized by the BDC 6.9 clone is in fact a hybrid peptide formed by the fusion of a previously described proinsulin C-peptide cleavage product, LQTLAL (Irminger *et al.* 1997), to a second peptide, NAARD (Delong *et al.* 2016). The NAARD peptide sequence corresponds to amino acids 55–59 in the mproIAPP₁₋₇₀ intermediate and is part of the C terminal fragment released following mproIAPP₁₋₇₀ cleavage by PC2 (Fig. 1B). The LQTLAL-NAARD hybrid peptide is present in mouse islet fractions, indicating that it is naturally formed by beta cells

**Figure 2**

IAPP epitopes in type 1 diabetes. Primary structures of mouse and human proIAPP peptides are shown. Colours denote N- and C-terminal flanking regions (blue), mature peptide (green), and residues removed by CPE and PAM during processing (grey). Epitopes are indicated in solid lines. Broken lines indicate residues within the peptide that form autoantigens following additional modifications. (A) The primary structure of NOD and Balb/c mproIAPP₁₋₇₀: epitope mIAPP₁₋₂₀, originally termed KS20, is present in the pro and mature peptide forms; an additional autoantigen is formed in NOD mice by fusion between the mproIAPP₅₅₋₅₉ sequence within the C-terminal-flanking region, and a C-peptide cleavage product (purple). Balb/c mice form a non-antigenic hybrid peptide due to a single amino acid substitution in the C-terminal-flanking region of mproIAPP. (B) The primary structure of the hpreproIAPP signal peptide (orange) and hproIAPP₁₋₆₇: epitopes hpreproIAPP₅₋₁₃ and hpreproIAPP₉₋₁₇ are present in the signal peptide; two autoantigens are formed by hybrid peptides derived from the N- and C-terminal flanking regions of hproIAPP₁₋₆₇ fused with a C-peptide cleavage product (purple); hproIAPP₄₂₋₆₂ forms an additional epitope following citrullination of R51 and R59 (red asterisks). This epitope has also been termed IAPP₆₅₋₈₄(73-Cit,81-Cit) based on its position in the hpreproIAPP peptide.

(Delong *et al.* 2016). Moreover, LQTLAL-NAARD-reactive CD4 T cells are present in NOD mouse pancreas prior to and following diabetes onset (Wiles *et al.* 2017). A second, independent pathogenic T cell clone, BDC 9.3, was also found to react to this same hybrid peptide (Delong *et al.* 2016). In BALB/c mice, an R58G substitution in mproIAPP renders the hybrid peptide only weakly immunogenic (Wiles *et al.* 2017), thus accounting for the original findings that localized the NOD-specific autoantigen to the *Iapp* locus (Dallas-Pedretti *et al.* 1995). With respect to humans, the equivalent fusion peptide (GQVELGGG-NAVEVLK) is formed by parts of human C-peptide and the C-terminal fragment of hproIAPP (spanning hproIAPP₅₂₋₅₈) (Fig. 2B). When presented on major histocompatibility complex class II (MHCII), encoded by the high-risk human leukocyte antigen (HLA) allele DQ8 (DQA1*03:01; DQB1*03:02), GQVELGGG-NAVEVLK activated two CD4 T cell clones expanded from a recent-onset T1D subject (Delong *et al.* 2016), suggesting that hybrid peptide epitopes containing IAPP regions may be of importance in human T1D. Other peptides fused to C-peptide fragments also appear to form hybrids recognized by autoreactive T cells in NOD mice and/or humans, including C-peptide

fragments fused with neuropeptide Y or chromogranin A fragments (Babon *et al.* 2016, Delong *et al.* 2016). Along with the GQVELGG-NAVEVLK hybrid peptide, an additional hproIAPP-derived hybrid autoantigen has been identified in a T1D patient (Babon *et al.* 2016), consisting of a fusion between C-peptide-derived LQTLAL and hproIAPP₁₋₇ (Fig. 2B).

Additional epitopes derived from IAPP and its preproIAPP precursor have been identified in human T1D subjects and NOD mice. The NOD diabetogenic T cell line BDC 5.2.9 was found to react to a 20 amino acid region, originally termed KS20 and referred to here as mIAPP₁₋₂₀, corresponding to residues 1–20 of the mature mIAPP₁₋₃₇ peptide (Delong *et al.* 2011) (Fig. 2A). CD4 T cells reactive to mIAPP₁₋₂₀ MHCII tetramers are present in the pancreases of ~40% of pre-diabetic NOD and >80% of diabetic NOD mice (Baker *et al.* 2013). Two additional mIAPP₁₋₂₀ reactive CD4 T cell lines (BDC-5/S34 and BDC-9/S3.5) were also cloned from the original BDC5 and BDC9 T cell lines, isolated from different NOD mice. Whether the equivalent region in hIAPP₁₋₂₀ is also an epitope in human T1D remains to be investigated. CD4 T cells derived from T1D islets recognize another

IAPP-derived epitope in humans, corresponding to residues hproIAPP_{42–62}, in which amino acids R51 and R59 have been post-translationally modified to citrulline residues (Babon *et al.* 2016) (Fig. 2B). This raises the further possibility that altered post-translational modification of IAPP and its precursor forms could yield additional beta cell autoantigens.

IAPP epitopes targeted by CD8 T cells

We identified two regions derived from the signal peptide of hpreproIAPP as epitopes, hpreproIAPP_{5–13} and hpreproIAPP_{9–17} (Fig. 2B), which activate cytotoxic CD8 T cells from T1D subjects (Panagiotopoulos *et al.* 2003, Ouyang *et al.* 2006, Standifer *et al.* 2006). These two hpreproIAPP epitopes bind to HLA-A*0201, a common HLA Class I allele that confers additional T1D risk in patients carrying high-risk HLA Class II alleles (Fennessy *et al.* 1994, Robles *et al.* 2002). Both of these epitopes appear to associate with T1D; hpreproIAPP_{5–13} elicited a response in CD8 T cells from six of nine recent-onset T1D subjects carrying the HLA-A*0201 allele (Panagiotopoulos *et al.* 2003), and hpreproIAPP_{5–13} autoreactive CD8 T cells are increased in recent-onset HLA-A2-positive T1D subjects relative to non-diabetic HLA-A2 sibs (Velthuis *et al.* 2010). hpreproIAPP_{9–17}-reactive CD8 T cells were also elevated in T1D subjects and GAD2 autoantibody-positive first-degree relatives compared to autoantibody-negative subjects (Standifer *et al.* 2006). Autoreactive T cell populations decline following diabetes onset (Trudeau *et al.* 2003, Coppieters *et al.* 2012), presumably due to lack of antigen following substantive beta cell loss. Consistent with this, hpreproIAPP_{5–13}-reactive peripheral blood mononuclear cells (PBMCs) were diminished in HLA-A*0201-positive T1D subjects with long-standing diabetes (>180 days) (Panagiotopoulos *et al.* 2003). The idea that signal peptide-derived peptides could generate potential epitopes in T1D has generated some interest, and indeed an epitope derived from the signal peptide region of proinsulin has also been described (Skowera *et al.* 2008). Signal peptides are rich in hydrophobic amino acids that could facilitate binding to the HLA-A*0201 cleft, and, because they are endoplasmic reticulum (ER) resident, can also bypass transporter associated with antigen processing (TAP)-dependent processing (Henderson *et al.* 1992, Wei & Cresswell 1992, Panagiotopoulos *et al.* 2003). Ultimately, studies in larger patient and at-risk (autoantibody positive) populations, including those early in disease pathogenesis, will be necessary to determine the role of hpreproIAPP-derived epitopes in T1D.

ProIAPP processing and IAPP epitopes

The identification of multiple IAPP-derived autoepitopes resulting from signal peptides, hybrid peptides and post-translational modifications raises the possibility that IAPP and its precursor forms may be processed differently in the presence of beta cell dysfunction in early autoimmunity. Indeed, we recently reported disproportionately elevated levels of the proIAPP processing intermediate hproIAPP_{1–48} in T1D (Courtade *et al.* 2017a) suggesting that, along with impaired proinsulin processing (Roder *et al.* 1994, Hartling *et al.* 1997, Truyen *et al.* 2005, Mirmira *et al.* 2016, Sims *et al.* 2016, Watkins *et al.* 2016, Rodriguez-Calvo *et al.* 2017), impaired proIAPP processing may be a characteristic of beta cells in T1D. While IAPP is normally secreted relative to insulin at a molar ratio of approximately 1:100 (Kautzky-Willer *et al.* 1994, Dechenes *et al.* 1998, Kahn *et al.* 1998, Knowles *et al.* 2002, Hull *et al.* 2004), the ratio of IAPP:insulin secretion or expression may increase in states of beta cell dysfunction (O'Brien *et al.* 1991, Pieber *et al.* 1993, Hiramatsu *et al.* 1994, Mulder *et al.* 1995a, 1996, Ahrén & Gutniak 1997, Hull *et al.* 2004, Krizhanovskii *et al.* 2017). Interestingly, a small study from the Better Diabetes Diagnosis cohort revealed that ~11% of children with T1D had markedly elevated plasma IAPP levels relative to C-peptide and pro-insulin levels at diagnosis (Paulsson *et al.* 2014). An increase in the ratio of IAPP:insulin or aberrant IAPP and insulin processing in secretory granules during beta cell dysfunction may promote accumulation of antigenic IAPP cleavage products and increase the probability of forming antigenic hybrid peptides. Collectively, the above findings indicate that IAPP is likely a common autoantigen in T1D, but its importance in disease pathogenesis remains to be determined.

IAPP aggregation and islet inflammation

The propensity of IAPP to aggregate and form islet amyloid renders it deleterious to beta cells. As IAPP aggregates, it first forms small soluble aggregate species, also termed oligomers, and subsequently proceeds to assemble into ordered beta-sheet-rich fibrils that amass into visible amyloid deposits. While fibrils are relatively inert, soluble pre-fibrillar aggregate species are cytotoxic to beta cells. In addition, recent evidence has established IAPP aggregates as potent proinflammatory stimuli. In this section, we discuss the role of IAPP aggregates in islet inflammation, using the term pre-fibrillar aggregates to describe species of soluble IAPP aggregates that populate the lag phase

of IAPP fibrillation, from early formed, small oligomeric species to larger, soluble aggregates that have not yet formed fibrils. Through activation of pattern recognition receptors, pre-fibrillar IAPP aggregates induce the release of a milieu of pro-inflammatory cytokines from innate immune cells, including interleukin-1 beta (IL1B) and tumour necrosis factor alpha (TNF), both of which are known to induce beta cell dysfunction and death. IAPP-induced islet inflammation seems increasingly likely to play a role in T2D pathogenesis (reviewed in Westwell-Roper *et al.* 2014a and Eguchi & Nagai 2017); little is known about whether IAPP aggregates form and induce inflammation in T1D.

IAPP aggregates induce islet inflammation

The inflammatory nature of IAPP aggregates appears to be a major contributor to chronic sterile inflammation of pancreatic islets. Much of this evidence has been garnered from hIAPP transgenic rodents, which express the hIAPP sequence under control of the rat insulin promoter to drive hIAPP overexpression in beta cells. Beta cell dysfunction accompanied by secretory demand such as that induced by high-fat diet or genetic obesity, leads to hIAPP aggregation and islet amyloid formation (which does not otherwise occur in wild-type rodents expressing only rIAPP). Expression of inflammatory cytokines and chemokines is elevated in islets of hIAPP transgenic mice; hIAPP transgenic islets express and secrete chemokines CXCL1 and CCL2 and have increased expression of macrophage markers *Itgam* and *Adgre1*, as well as inflammatory genes *Il1b* and *Nlrp3* (Westwell-Roper *et al.* 2011, 2014b, 2016). Moreover, hIAPP transgenic mice fed a high-fat diet for 12 months have dramatically elevated proinflammatory gene expression in islets including *Ccl2*, *Cxcl1*, *Nlrp3*, *Pycard*, *Casp1*, *Il1b*, *Tnf*, *Il6*, *Adgre1* and *Itgax* (Meier *et al.* 2014), the expression of which is strikingly higher than the effect of high-fat diet alone, supporting the idea that IAPP aggregates are a major – and perhaps essential – trigger for islet inflammation. Consistent findings have recently been reported in cultured human islets, demonstrating that islet amyloid formation correlates with increased IL1B and decreased levels of endogenous IL1 receptor antagonist, IL1RN (Hui *et al.* 2017). Furthermore, inhibition of hIAPP expression or aggregation in human islets reduced IL1B levels (Park *et al.* 2017).

Within islets, the primary source of inflammatory cytokines in response to IAPP aggregates is islet macrophages, and these cells appear to play an integral

role in islet amyloid clearance and the ensuing deleterious inflammatory response. Though IL1B synthesis has been demonstrated in most cell types, and reports suggest that beta cells can produce IL1B under certain conditions (Ribaux *et al.* 2007, Böni-Schnetzler *et al.* 2008), we found that clodronate-mediated depletion of islet macrophages *in vivo* virtually ablates islet *Il1b* and *Tnf* expression in hIAPP transgenic mice (Westwell-Roper *et al.* 2014b). These data indicate that macrophages are likely the major, if not sole, source of inflammatory cytokines within the islet. Extracellular islet amyloid, localized in the perivascular space between the beta cell basement membrane and islet capillaries, is ideally situated to interact with resident islet macrophages residing next to intraislet blood vessels (Hume *et al.* 1984, Calderon *et al.* 2008). Islet macrophages containing IAPP immunoreactive lysosome-like structures were first described in human insulinomas, monkey pancreatic islets and hIAPP transgenic mouse islets by de Koning and colleagues (1994). Similarly, we found macrophages within hIAPP transgenic mouse islets frequently in close association with, and appearing to phagocytose, amyloid deposits (Westwell-Roper *et al.* 2011, 2014b). Depletion of macrophages in hIAPP transgenic mice improves glycemia while alleviating expression of inflammatory cytokines, pointing to an important role for macrophages in amyloid-induced beta cell dysfunction. Interestingly, macrophage depletion increased amyloid severity (Westwell-Roper *et al.* 2014b), suggesting that islet macrophages may play a role in islet amyloid clearance.

IAPP aggregates, due to their tendency to induce secretion of CCL2 and other chemokines from islets, likely recruit additional macrophages to islets. Indeed, we found hIAPP treatment of human islets to have a concentration-dependent chemotactic effect on THP-1 cells (Westwell-Roper *et al.* 2011). Consistent with this, the number of F4/80+ macrophages is increased in endogenous and transplanted islets of hIAPP-expressing mice compared to those of wild-type controls (Westwell-Roper *et al.* 2011, Meier *et al.* 2014). In contrast, de Koning and colleagues found that macrophage density was not different between islets with and without amyloid in diabetic humans and *Macaca mulatta* monkeys, nor between hIAPP transgenic and control mice (de Koning *et al.* 1998). In a more recent study, amyloid-positive islets in T2D were found to have significantly more macrophages (CD68+ cells) accumulating within or near amyloid plaques and around microvessels relative to matched T2D subjects without amyloid and to non-diabetic controls (Kamata *et al.* 2014). The majority of CD68+ cells in this study co-expressed nitric oxide synthase 2 (NOS2), whereas the number of

CD204+ CD163+ macrophages (indicative of an M2-like alternatively activated phenotype) was unaltered, suggesting that hIAPP specifically augments a population of pro-inflammatory macrophages in islets. Thus, while macrophage phenotype appears to be a major driver of amyloid-induced islet inflammation, the importance of macrophage number remains to be determined.

The pro-inflammatory effects of hIAPP aggregates on isolated innate immune cells can be clearly observed *in vitro*. Some of the earliest evidence was provided by a study showing that hIAPP, but not rIAPP, augmented ionophore-induced colony stimulating factor (CSF2) and leukotriene C4 release from human eosinophils, though it was unclear whether this was due to general toxicity of hIAPP aggregates (Hom *et al.* 1995). Subsequently, hIAPP was reported to induce the release of a number of cytokines, including IL1B, TNF, IL6 and IL8, in murine microglia and human monocyte and astrocyte cell lines, effects that were not exerted by rIAPP (Gitter *et al.* 2000, Yates *et al.* 2000). Subsequent studies by our group and others have more precisely defined the mechanisms by which hIAPP aggregation induces inflammation. Masters and colleagues demonstrated that overnight incubation of lipopolysaccharide (LPS)-primed bone marrow-derived dendritic cells (BMDCs) with hIAPP induced processing and secretion of IL1B by activating the NLR family pyrin domain containing 3 (NLRP3) inflammasome (Masters *et al.* 2010). We found that even in the absence of a priming stimulus, hIAPP induced a broad pro-inflammatory profile in bone marrow-derived macrophages (BMDMs), marked by increased expression and secretion of TNF, IL1A, IL1B as well as numerous chemokines including CCL2, CCL3, CXCL1, CXCL2 and CXCL10 (Westwell-Roper *et al.* 2011). The lack of any inflammatory action of rIAPP in these studies clearly shows that macrophage recruitment and activation is a property of IAPP aggregates rather than a biological activity of IAPP monomers. Consistent with this, application of the amyloid-binding dye Congo Red prevents hIAPP-induced inflammasome activation in BMDMs (Sheedy *et al.* 2013), likely by either inhibiting hIAPP aggregation or possibly binding to and rendering aggregates non-toxic. Likewise, Congo Red reduced IL1B levels in cultured human islets (Park *et al.* 2017). Furthermore, inhibition of the cognate receptor for monomeric IAPP with the IAPP antagonist AC187 does not reduce hIAPP-induced IL1B secretion in primed cells (Masters *et al.* 2010). Collectively, these data reveal that hIAPP aggregation elicits a robust pro-inflammatory response in innate immune cells, and further suggest that hIAPP can provide

both the priming and activation stimulus for secretion of the pro-inflammatory cytokine IL1B.

Mechanism of IAPP uptake and NLRP3 inflammasome activation

hIAPP-induced IL1B release from BMDMs and BMDCs is dependent on phagocytosis of hIAPP aggregates and activation of NLRP3 and caspase 1 (CASP1). Deletion of either *Nlrp3* or *Casp1* abrogates hIAPP-induced IL1B secretion in cells, regardless of whether they are first primed with LPS or hIAPP aggregates (Masters *et al.* 2010, Sheedy *et al.* 2013, Westwell-Roper *et al.* 2016). Furthermore, pharmacological inhibition of NLRP3 with glyburide inhibited hIAPP-induced IL1B secretion (Masters *et al.* 2010, Westwell-Roper *et al.* 2011). Though the mechanism of NLRP3 activation by hIAPP has not been fully elucidated, phagocytosis of hIAPP aggregates and accumulation of hIAPP fibrils within the phagolysosome system appear to play a critical role. Accumulation of IAPP immunoreactivity in macrophages occurs through a phagocytosis-dependent pathway (Badman *et al.* 1998), and IL1B release is attenuated by inhibition of phagocytosis with cytochalasin D (Masters *et al.* 2010, Westwell-Roper *et al.* 2011, Sheedy *et al.* 2013). Extracellularly applied hIAPP appears intracellularly in cultured macrophages as early as 1 h following incubation and continues to increase up to 6 h, with IAPP co-localizing with macrophage marker CD11b, a late endosome/lysosome marker (Badman *et al.* 1998). After 6 h of incubation, IAPP immunoreactivity can be found loosely packed in phagosome-like organelles, and from 24 to 72 h, IAPP-reactive fibrils accumulate in dense lysosome-like organelles. This timeline is consistent with that of IL1B release in hIAPP-treated cells: secretion of IL1B in unprimed or LPS-primed macrophages appears after approximately 6 h of incubation and continues thereafter (Masters *et al.* 2010, Sheedy *et al.* 2013, Westwell-Roper *et al.* 2016). The requirement for longer incubation for maximal IL1B secretion indicates that the process of NLRP3 activation by hIAPP aggregates is different from that of ligands like ATP, which activate the inflammasome more rapidly.

Within phagolysosomes, hIAPP appears to form fibrils. This is evidenced by small fibrillar structures present in lysosome structures (Badman *et al.* 1998) and the accumulation of thioflavin S, which binds amyloid fibrils (Sheedy *et al.* 2013). Knockout of the scavenger receptor CD36 abrogates the accumulation of the thioflavin S signal in BMDMs and attenuates IL1B secretion, indicating its involvement in seeding aggregates within

the phagolysosome system, and that the accumulation of fibrils is necessary for maximal NLRP3 activation (Sheedy *et al.* 2013). IAPP fibrils appear to persist in lysosomes even following a 72-h washout (Badman *et al.* 1998), suggesting that macrophages are unable to break down and extrude internalized fibrils. This may lead to phagolysosomal disruption, a known activation mechanism for the NLRP3 inflammasome. Thioflavin S accumulation in hIAPP-treated phagocytes is associated with leakage of fluorescent dextran into the cytoplasm (Sheedy *et al.* 2013). Furthermore, inhibition of cathepsin B, which is released during lysosomal disruption, attenuates hIAPP-induced IL1B secretion in LPS-primed cells (Masters *et al.* 2010, Westwell-Roper *et al.* 2011, Sheedy *et al.* 2013) and inhibition of lysosomal acidification with bafilomycin A1 attenuates hIAPP-induced IL1B secretion in LPS-primed BMDs and BMDMs (Masters *et al.* 2010, Sheedy *et al.* 2013). Collectively, these data suggest that macrophages phagocytose hIAPP aggregates, and may not effectively extrude accumulating fibrils in lysosomes, resulting in the activation of an inflammatory response (Fig. 2).

IAPP priming of macrophages

In addition to activating the NLRP3 inflammasome, hIAPP aggregates can also provide the priming signal leading to induction of nuclear factor kappa B subunit 1 (NFKB1) signalling and synthesis of pro-IL1B and other cytokines (Fig. 2). Application of aggregating concentrations of hIAPP to unprimed BMDMs induces expression of pro-inflammatory cytokines including *Il1b*, *Il1a*, *Tnf* and *Il6*. Furthermore, hIAPP-primed cells secrete numerous cytokines and chemokines, which do not require inflammasome activation, including IL1A, TNF, IL6, IL-10, CCL2, CCL3, CCL4, CCL5, CXCL1, CXCL2 and CXCL10 (Westwell-Roper *et al.* 2011, 2016). Importantly, this occurs under conditions and hIAPP concentrations that do not induce cell death, nor is the effect of hIAPP aggregates inhibited by polymyxin, indicating that it cannot be explained by contamination of hIAPP peptide preparations with LPS (Westwell-Roper *et al.* 2011). This is consistent with the findings of Yates and colleagues who showed that application of hIAPP to unprimed THP-1 cells rapidly induced the expression of *Il1b* and *Tnf* (Yates *et al.* 2000). Like activation of the inflammasome, hIAPP but not rIAPP induces cytokine expression and NFKB1 signalling, indicating that priming is aggregation dependent (Westwell-Roper *et al.* 2011, 2016).

We have reported that activation of NFKB1 and priming by hIAPP is dependent on toll-like receptor (TLR)

2 or a TLR 2/6 heterodimer and myeloid differentiation primary response 88 (MYD88)-dependent signalling. hIAPP induction of NFKB1 activity and pro-inflammatory cytokines is abrogated in *Tlr2*^{-/-} and *Myd88*^{-/-} BMDMs, and in a human TLR2-expressing reporter cell line treated with TLR2- or TLR6-neutralizing antibodies (Westwell-Roper *et al.* 2016). Furthermore, *Tlr2* knockout islets express less *Il1b* in response to hIAPP application, and immunoneutralization of TLR2 reduces *Il1b* expression in islets from hIAPP transgenic rodents (Westwell-Roper *et al.* 2016). Though hIAPP can act as a priming stimulus, it is notably less potent than LPS; LPS priming results in a 10-fold greater induction of TNF relative to hIAPP over 24 h (Westwell-Roper *et al.* 2011). Moreover, LPS-primed cells subsequently treated with hIAPP secrete approximately 10-fold greater levels of IL1B than cells treated with hIAPP only (Westwell-Roper *et al.* 2016); this may explain why others have not observed robust IL1B secretion from cells stimulated with hIAPP alone compared to LPS-primed cells treated with hIAPP (Masters *et al.* 2010, Sheedy *et al.* 2013). The relative contributions of hIAPP-induced NFKB1 and NLRP3 activation in islet inflammation and ensuing beta cell dysfunction *in vivo* remain to be tested, though IL1 clearly plays a critical role, as IL1 antagonism ameliorates inflammation, and beta cell dysfunction and death, in hIAPP transgenic mice and isolated human islets (Westwell-Roper *et al.* 2011, 2015, Hui *et al.* 2017, Jin *et al.* 2017, Park *et al.* 2017). Furthermore, though occurring through distinct mechanisms, both priming and NLRP3 activation by IAPP are likely to perpetuate a feed-forward inflammatory activation in macrophages; we found that IL1 signalling potentiates further IAPP-induced IL1B secretion (Westwell-Roper *et al.* 2011), and IL1B has also been shown to enhance islet amyloid formation in both human and hIAPP transgenic islets (Park *et al.* 2017).

Inflammatory IAPP species

While both the priming and activating signals provided by IAPP are dependent on its ability to aggregate, the precise aggregate species that induce maximal inflammatory responses have not been defined. Since aggregation of hIAPP progresses *in vitro* from monomeric through to pre-fibrillar aggregates and subsequently fibrillar forms over the course of minutes to hours (depending on the concentration and solvent used), application of freshly dissolved hIAPP to macrophages will expose these cells to a range of hIAPP aggregate species. Early, pre-fibrillar hIAPP species are the most pro-inflammatory (Masters *et al.* 2010, Westwell-Roper *et al.* 2011, 2016) and most

toxic to INS1 cells (Abedini *et al.* 2016). It should be noted that fibrillar hIAPP species can, however, activate the inflammasome in LPS-primed macrophages, though to a lesser extent than freshly dissolved hIAPP (Westwell-Roper *et al.* 2016). Furthermore, through size fractionation, Masters and colleagues identified that smaller pre-fibrillar hIAPP aggregate species of <100nm induced the highest level of IL1B secretion from BMDCs after overnight incubation (Masters *et al.* 2010). Though aggregation is necessary for priming and activation of macrophages, it should also be noted that monomeric forms of IAPP may also influence macrophage function. Rodent IAPP as well as the related peptide calcitonin related polypeptide alpha (CALCA) have both been shown to increase phagocytic activity in murine peritoneal macrophages through a cyclic AMP-dependent pathway (Ichinose & Sawada 1996). We observed that both hIAPP and rIAPP enhance BMDM viability, raising the possibility that both monomeric and aggregate forms may provide survival signals to BMDMs (Westwell-Roper *et al.* 2011). Thus, the process of hIAPP

aggregation from monomers and through early pre-fibrillar aggregates to mature fibrils, exposes macrophages to a series of cues that may maximize both inflammasome priming and activation; through binding of aggregates by TLRs to disruption of phagolysosomes by accumulating aggregates and/or fibrils (Fig. 3).

Could IAPP-induced inflammation play a role type 1 diabetes?

Given the role of macrophages and islet inflammation in initiating T1D (Oschilewski *et al.* 1985, Charlton *et al.* 1988, Lee *et al.* 1988a,b, Chung *et al.* 1997, Jun *et al.* 1999a,b, Calderon *et al.* 2006), it seems plausible that hIAPP aggregates, via induction of islet inflammation, could be a trigger or accelerator of autoimmunity in T1D. Amyloid has not been thoroughly investigated in T1D pancreas, and the deficiency of IAPP (along with insulin) associated with beta cell loss makes development of extensive amyloid plaques in T1D seem unlikely. However, amyloid

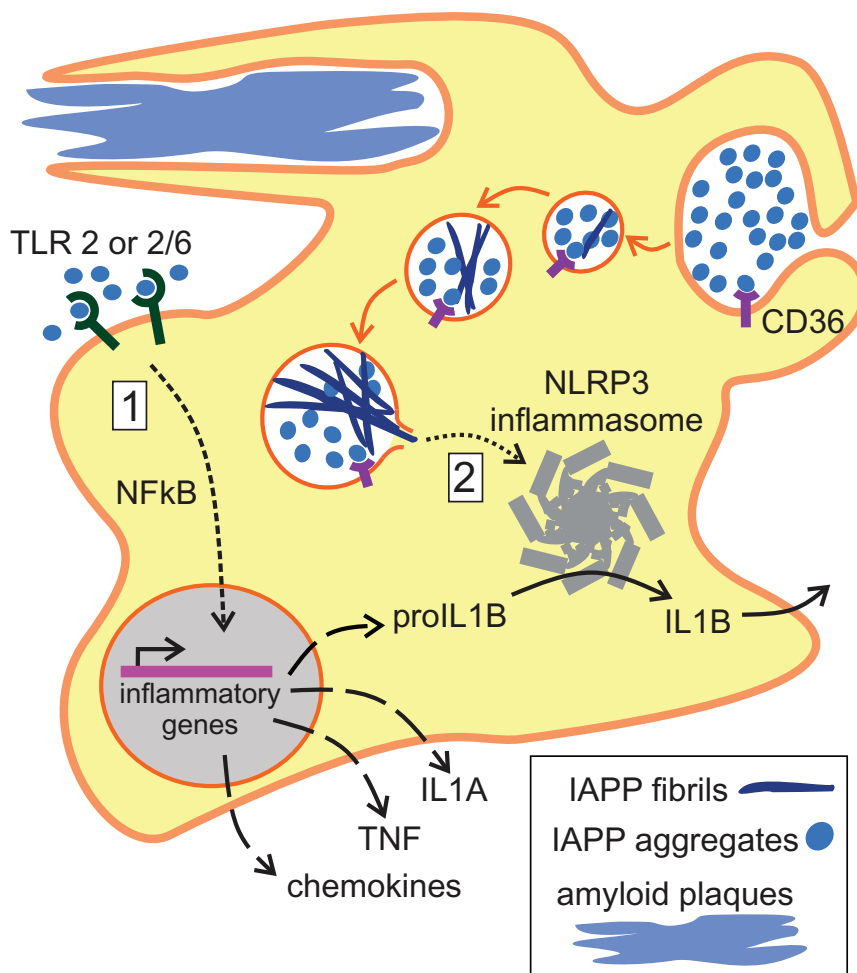


Figure 3

Macrophage activation by IAPP. Prefibrillar IAPP aggregates may signal through toll-like receptor (TLR) 2 or a TLR2/6 heterodimer to activate NFκB signalling and provide signal 1 (indicated) for proIL1B synthesis. IAPP aggregates are also taken up through phagocytosis and accumulate in the phagolysosomal pathway where they may seed to form fibrils, possibly mediated via the scavenger receptor CD36. Accumulation of IAPP aggregates and/or fibrils results in lysosomal disruption and the release of cathepsin B, providing signal 2 (indicated) for NLRP3 activation and inflammasome assembly. The NLRP3 inflammasome processes proIL1B to mature IL1B, which is subsequently secreted. Large extracellular deposits of mature IAPP fibrils and/or amyloid plaques may also induce frustrated phagocytosis resulting in further inflammation.

was recently reported in two of six pancreas biopsies from recent onset T1D subjects (Westermarck *et al.* 2017), and it remains possible that early pre-fibrillar aggregates, which are difficult to observe histologically (Zraika *et al.* 2012), might be present early in disease. This is plausible given that beta cell dysfunction increase IAPP:insulin ratios (O'Brien *et al.* 1991, Pieber *et al.* 1993, Hiramatsu *et al.* 1994, Mulder *et al.* 1995a,b, 1996, Ahrén & Gutniak 1997) and impairs proIAPP processing (Courtade *et al.* 2017a), and beta cell dysfunction is thought to occur early in T1D (Roder *et al.* 1994, Hartling *et al.* 1997, Keskinen *et al.* 2002, Truyen *et al.* 2005, Ferrannini *et al.* 2010, Rodriguez-Calvo *et al.* 2017). As TLR signalling and the NLRP3 inflammasome have been implicated in T1D pathogenesis (Tai *et al.* 2016), it follows that, if pre-fibrillar hIAPP aggregates form in T1D, the inflammatory properties of hIAPP aggregates might also play a role in T1D pathology.

IAPP replacement in type 1 diabetes

Beta cells are the major source of circulating IAPP, and their loss in T1D results in deficiency of both circulating insulin and IAPP. IAPP levels are reduced in T1D subjects and negatively correlate with disease duration, as is the case for T2D subjects requiring exogenous insulin therapy (Harterter *et al.* 1990, Koda *et al.* 1992, Akimoto *et al.* 1993, Heptulla *et al.* 2005, Huml *et al.* 2011). Thus, it has been postulated that IAPP deficiency may contribute to the metabolic dysregulation of established T1D. However, *Iapp* knockout mice have a subtle metabolic phenotype (Gebre-Medhin *et al.* 1998, Olsson *et al.* 2012), suggesting only a modest role of IAPP in glucose homeostasis. Nevertheless, *Iapp* knockout mice with alloxan-induced diabetes have slightly more severe hyperglycemia than wild-type controls (Mulder *et al.* 2000), indicating that, to some extent, IAPP loss may exacerbate metabolic dysregulation in insulin-deficient states.

Many studies have investigated the effects of IAPP replacement in T1D using pramlintide, an analogue of IAPP that is similar to the non-aggregating rodent form of IAPP, containing three proline substitutions which prevent aggregation. Pramlintide injection has no influence on glycemia in the fasting state, nor during experimental hyperinsulinemic–euglycemic or hyperinsulinemic–hypoglycemic challenge (Kolterman *et al.* 1996, Nyholm *et al.* 1996, Amiel *et al.* 2005). However, single-dose pramlintide injection in conjunction with insulin prior to a meal more effectively attenuates postprandial glycemia than insulin alone in T1D subjects (Fineman *et al.* 2002, Heptulla *et al.* 2005, 2009, Chase *et al.* 2009,

Hassan & Heptulla 2009, Weinzimer *et al.* 2012). Pramlintide and insulin co-administration was also shown to be more effective relative to higher doses of insulin alone, pointing to distinct metabolic actions of IAPP and insulin (Heptulla *et al.* 2005). Improved postprandial glycemia with single-dose pramlintide supplementation involves the inhibition of postprandial glucagon secretion and delayed gastric emptying. Relative to insulin therapy alone, single-dose pramlintide supplementation represses postprandial glucagon secretion in T1D subjects (Fineman *et al.* 2002, Heptulla *et al.* 2005, Chase *et al.* 2009, Hassan & Heptulla 2009). Furthermore, pramlintide administration delays gastric emptying of both solids and liquids in T1D subjects (Kong *et al.* 1997, 1998, Vella *et al.* 2002, Woerle *et al.* 2008, Chase *et al.* 2009). In fact, IAPP deficiency may contribute to the accelerated gastric emptying observed in uncomplicated T1D (Vinik *et al.* 2008, Woerle *et al.* 2008). These findings indicate that IAPP limits postprandial glycemic and glucagon excursions.

In addition to its metabolic effects, preclinical studies have demonstrated that IAPP has anorectic effects on food intake; these actions are dependent on IAPP-responsive neurons in the brain, particularly within the area postrema (Edwards *et al.* 1998, Lutz *et al.* 2010) and are not due to malaise or reduced water intake (Lutz *et al.* 1995, Morley *et al.* 1996). In humans, pramlintide reduces food intake in both T1D and control subjects (Asmar *et al.* 2010) and induces sustained weight loss in T1D subjects (Whitehouse *et al.* 2002, Ratner *et al.* 2005, Edelman *et al.* 2006). However, given the modest phenotype of IAPP-deficient animals, physiologically IAPP is likely a minor contributor to overall satiety and may act to fine-tune the more robust effects of other satiety signals.

Clinical use of pramlintide for type 1 diabetes

In 2005, pramlintide (marketed as Symlin) became the first injectable approved by the US FDA for the treatment of T1D since insulin and has been suggested for supplementation with meal-time insulin to improve postprandial hyperglycemia in both T1D and T2D. In several clinical trials conducted by Amylin Pharmaceuticals, meal-time pramlintide supplementation led to consistent and lasting weight loss, reductions in insulin requirements, as well as improved postprandial glycemia in T1D subjects on insulin (Whitehouse *et al.* 2002, Ratner *et al.* 2005, Edelman *et al.* 2006). The glycemic benefits of pramlintide supplementation occur regardless of disease duration (Herrmann *et al.* 2016), and pramlintide was also shown to improve glycemic control in T1D patients who are

approaching, but have not achieved, target glycemic control on insulin alone (Ratner *et al.* 2005).

Given the potential indications of pramlintide administration in T1D, one could reasonably question why its use as an adjunct therapeutic to insulin has not had more uptake clinically; most clinical studies of pramlintide in diabetes were reported over a decade ago. Side effects of pramlintide administration are a likely reason; nausea, anorexia, vomiting and hypoglycemia are commonly reported adverse events (Whitehouse *et al.* 2002, Ratner *et al.* 2004, Edelman *et al.* 2006) and appear to be worse in T1D than in T2D or obese individuals. The anorexia and nausea induced by pramlintide may also be factors in sustained weight loss and long-term improvements in glycemia. The adverse side effects of pramlintide therapy occur early in the initiation of pramlintide therapy but reportedly lessen over time (Whitehouse *et al.* 2002, Ratner *et al.* 2004, Edelman *et al.* 2006). Insulin dose titration, gradual pramlintide dose escalation and changes to the mode of pramlintide administration, such as administering pramlintide at a ratio with meal-time insulin or as an infusion rather than injection, also appear to improve safety and tolerability (Edelman *et al.* 2006, Rodriguez *et al.* 2007, Kovatchev *et al.* 2008, Micheletto *et al.* 2013, Riddle *et al.* 2015). A better understanding of the biological actions of IAPP is clearly needed, along with further clinical study and mitigation of adverse events, before the potential benefit of pramlintide as an adjunct therapeutic to insulin can be realized. Indeed, more than three decades after the discovery of IAPP, understanding of its receptor – thought to be a heteromer of the calcitonin receptor with receptor activity modifying proteins RAMP1 or RAMP3 (Christopoulos *et al.* 1999) – and its intracellular signalling mechanism remains somewhat rudimentary.

IAPP aggregation and islet transplant dysfunction

IAPP aggregates may play an important role in islet graft failure. Islet amyloid has been found in human islets transplanted into diabetic immune-deficient mice (Westermarck *et al.* 1995, 1999, Finzi *et al.* 2005, Bohman & Westermarck 2012, Potter *et al.* 2015); hIAPP transgenic mouse islets transplanted into syngeneic or immune-deficient diabetic mice (Udayasankar *et al.* 2009, 2013, Westwell-Roper *et al.* 2011), and non-human primate islet allografts (Liu *et al.* 2012). In addition, biopsies of whole pancreas transplants and liver-engrafted islets obtained at autopsy from T1D subjects have demonstrated varying degrees of islet amyloid formation (Westermarck *et al.* 2008,

2012, León Fradejas *et al.* 2015). Despite being absent at the time of transplantation, islet amyloid forms rapidly in hIAPP transgenic mouse islets and human islets engrafted in diabetic mouse recipients, and its severity progresses over time (Westermarck *et al.* 2003, Udayasankar *et al.* 2009). Transplanted human islets in streptozotocin (STZ)-induced diabetic mice displayed amyloid as early as two weeks post-transplant (Westermarck *et al.* 1995, 1999); likewise, hIAPP transgenic mouse islets transplanted into syngeneic diabetic mice developed islet amyloid within one week post-transplant, which further progressed over 12 weeks (Udayasankar *et al.* 2009). In diabetic non-human primates transplanted with allogeneic islets, a minority of islet grafts were amyloid positive within four months, whereas islet amyloid was present in all grafts from recipients with longer-term graft survival (10–66 months) (Liu *et al.* 2012). To date, while only a small number of islet transplants in T1D recipients have been analyzed for islet amyloid, most have been found to have amyloid deposition (Westermarck *et al.* 2008, 2012). Thus, the prevalence of amyloid may be widespread in clinical islet transplants and warrants continued investigation.

Preclinical islet transplant models have revealed an association between islet amyloid formation and primary islet graft dysfunction and failure. hIAPP transgenic mouse islet grafts fail more rapidly than control islet grafts in diabetic syngeneic recipient mice, and amyloid severity within these islet grafts correlates with hyperglycemia and beta cell loss (Udayasankar *et al.* 2009, 2013). Similarly, in diabetic non-human primates receiving allogeneic or autologous islet transplants, amyloid severity in grafts correlated positively with hyperglycemia recurrence, and inversely with insulin positive area (Liu *et al.* 2012). This association is recapitulated in human islet transplants; we found that amyloid severity is associated with reduced beta cell area and graft failure following sub-optimal transplantation of human islets into immune-deficient, diabetic recipient mice (Potter *et al.* 2010). Whether islet amyloid contributes to clinical islet transplant failure requires further investigation. Westermarck *et al.* reported varying degrees of amyloid severity in 33–44% of engrafted islets in three out of four T1D recipients of clinical islet transplants (Westermarck *et al.* 2012); intriguingly, the patient in which no amyloid was observed had the lowest glycated hemoglobin levels of the four patients in this study. Islet amyloid was also reported to be present in most islets in two T1D recipients of whole pancreas allografts presenting with graft failure (León Fradejas *et al.* 2015), suggesting that amyloid may also contribute to failure of pancreas transplants. Clear demonstration of a causal role

for amyloid in islet graft failure awaits inhibitors of IAPP aggregation in preclinical models of islet transplantation.

The reason for rapid amyloid accumulation in islet grafts remains unclear. It appears to be independent of transplantation site, forming in islets engrafted under the kidney capsule, liver, or spleen of nude mice (Westermarck *et al.* 2003). It is possible that limited diffusion of secreted IAPP occurs prior to graft vascularization and perfusion, allowing time for IAPP accumulation and aggregation. However, this possibility is countered by the presence of islet amyloid in whole pancreas transplants (León Fradejas *et al.* 2015). We previously pointed out the similarities underlying islet dysfunction in T2D and following transplantation, and have proposed that the beta cell dysfunction in islet grafts predisposes to amyloid deposition, as in T2D (Potter *et al.* 2014). IAPP synthesis and secretion is disproportionately elevated (relative to insulin) in dysfunctional beta cells, including those following transplantation (Stadler *et al.* 2006, Rickels *et al.* 2008). We recently found elevated levels of the intermediate hproIAPP₁₋₄₈ in islet transplant recipients (Courtade *et al.* 2017a), suggesting that impaired proIAPP processing may be a characteristic of transplanted islets, as we and others have suggested may be the case for proinsulin (Davalli *et al.* 2008, Fiorina *et al.* 2008, Klimek *et al.* 2009). Because proinsulin:insulin ratios were higher in recipients of fewer islets (Klimek *et al.* 2009), it is possible that the secretory stress associated with sub-optimal mass islet transplantation exacerbates islet prohormone processing defects. Such a defect could contribute to amyloid formation and further worsen beta cell dysfunction, a possibility that is supported by our recent findings: islets from hIAPP transgenic mice lacking PC2 – and therefore having markedly impaired processing of the hproIAPP₁₋₄₈ intermediate – fail very rapidly following transplantation (Courtade *et al.* 2017b). Consistent with this, treatments that improve proIAPP processing and secretion of mature IAPP are associated with decreased amyloid formation and improved beta cell function in cultured human islets (Park *et al.* 2013). These findings also raise the possibility that beta cell prohormones may have value as biomarkers of graft function or impending graft failure.

Amyloid and islet transplant inflammation

A putative mechanism of amyloid-induced primary islet graft dysfunction is islet inflammation and elevated local concentrations of IL1B, owing to the potent inflammatory action of IAPP aggregates discussed above. We found that hIAPP transgenic islet grafts have increased

macrophage accumulation and *Il1b* expression relative to control grafts in diabetic, immune-compromised mouse recipients (Westwell-Roper *et al.* 2011). Furthermore, when recipients of hIAPP transgenic islet grafts were treated with the endogenous IL1 receptor antagonist, IL1RN, graft function and glycemic control were markedly improved (Westwell-Roper *et al.* 2011), suggesting an important role for amyloid-induced inflammation in graft dysfunction. In agreement with this, improved transplant outcomes were recently reported in immune-deficient mice that received transplants of IL1RN-treated islets from non-human primates (Jin *et al.* 2017).

Since inflammation may help trigger autoimmunity as well as allograft rejection, it is plausible that, in addition to contributing to primary graft dysfunction, amyloid-induced inflammation speeds immune-mediated loss of islet grafts. Most studies of islet amyloid and primary graft dysfunction have used immune-deficient or syngeneic recipients, limiting insight into whether islet amyloid might trigger islet allograft rejection. In one study of non-human primate islet allografts, Liu *et al.* found that when islet allograft rejection occurred rapidly due to insufficient immune suppression, islet amyloid was detected in only 1 of 6 recipients (Liu *et al.* 2012); however, it is plausible that early pre-fibrillar aggregates could have formed in islet grafts during this period. In addition, IAPP-derived autoantigens described above could have important implications for recurrent autoimmunity in clinical islet transplant recipients. Thus, the possibility that IAPP could accelerate immune-mediated graft loss via autoantigen presentation or aggregate-induced inflammation warrants investigation.

Considerations for beta cell replacement strategies

The importance of understanding the role of islet amyloid formation in islet graft failure has become more critical, as clinical islet transplantation and other beta cell replacement strategies show increasing promise as potentially curative treatments for T1D. Approaches to prevent or slow IAPP aggregation in transplanted islets have the potential to enhance graft survival and function. In fact, some therapeutic strategies intended to improve transplant outcomes may actually exacerbate IAPP aggregation and amyloid deposition. For example, immunosuppressive regimens currently used in clinical islet transplantation have direct deleterious effects on beta cells (Johnson *et al.* 2009), some of which may promote amyloid deposition. This is the case for glucocorticoids, including dexamethasone, though these are no longer

commonly used in islet transplants (Couce *et al.* 1996, Mulder *et al.* 1996). Another practice that may exacerbate islet amyloid formation in islet grafts is the use of heparin to prevent clotting during islet infusion. We found that the pre-treatment of islets with heparin enhances islet amyloid formation by promoting fibrillation of IAPP (Potter *et al.* 2015). Consequently, heparin pre-treatment was associated with poorer function of human islet grafts in immune-deficient mice. Encapsulation, a strategy being pursued to protect islet grafts from the recipient immune system, may further exacerbate islet amyloid formation and impair transplant outcomes (Bohman & Westermark 2012). In addition, putative adjunct therapies may influence islet amyloid formation in grafts. For example, the glucagon-like peptide 1 (GLP1) agonist exenatide was shown to increase plasma IAPP levels in T1D subjects with islet allograft dysfunction (Froud *et al.* 2008). While GLP-1 agonism increases secretion of IAPP and potentiates amyloid formation, it also alleviates the toxic effects of IAPP on beta cells (Aston-Mourney *et al.* 2011). In cultured human islets, exenatide was shown to improve islet function and survival, improving proIAPP processing thereby restoring mature IAPP secretion, and reduce IAPP aggregation (Park *et al.* 2013). Additional factors such as recipient diet and sex could have significant influences on islet amyloid formation in transplants; high fat diet was shown to augment amyloid formation in human islet grafts in immune-deficient mice (Dai *et al.* 2016), and in both preclinical animal models and human T2D, males tend to have more extensive pancreatic islet amyloid formation than females (Verchere *et al.* 1996, Zhao *et al.* 2008).

Alternative sources of beta cells are being pursued as more cost-effective and unlimited sources for transplantation, and these cells also need to be investigated for their propensity to form amyloid following transplantation. One potential source of islets for transplantation is pigs. We have previously shown that porcine IAPP differs from hIAPP by 10 amino acids, and as such, is non-amyloidogenic and non-toxic (Potter *et al.* 2010). Our finding that pig islet transplants lack any detectable amyloid may be one reason why porcine islets survive and function well following transplant. As another alternative beta cell source, glucose-responsive insulin producing cells can now be fully differentiated *in vitro* from human embryonic stem cells (hESCs) and show great promise for transplantation (Pagliuca *et al.* 2014, Rezania *et al.* 2014). As observed by Rezania *et al.* differentiated insulin-producing cells express IAPP, albeit at lower levels than adult human islets,

and thus whether amyloid occurs in hESC-derived cell transplants needs to be examined.

Conclusions

Though our current understanding of IAPP's role in T1D is limited, the evidence reviewed here suggests that IAPP could potentially play an immunomodulatory role in diabetes progression, while the lack of IAPP in established diabetes may have glucoregulatory consequences. Opportunities for intervention of T1D exist at multiple disease stages: prevention of autoimmunity; slowing or reversing of beta cell loss prior to clinical onset of diabetes; and improving glycemic control after diabetes onset via injectable or cell-based therapies. IAPP could be a putative target at each of these intervention stages. Targeting IAPP to prevent or slow T1D progression prior to clinical onset is an avenue that warrants further evaluation, as IAPP-derived autoantigens or IAPP aggregate-induced inflammation may accelerate beta cell autoimmunity and beta cell loss. In individuals with established T1D, IAPP replacement has been investigated as an adjunct to insulin to improve glycemic control, but with limited success. On the other hand, while beta cell replacement strategies continue to show great promise as a potentially curative treatment for T1D, IAPP aggregation likely limits transplanted beta cell survival and function; thus, efforts to elucidate the factors that influence aggregate formation in this setting (as in T2D), and strategies to circumvent this, are warranted. Finally, as the pathogenesis of T1D is still poorly defined but involving both beta cell dysfunction and breakdown of immune tolerance, exploring the potential utility of proIAPP intermediates as biomarkers for beta cell dysfunction could be important to inform other possible therapeutic strategies and to better understand this complex and increasingly prevalent disease.

Declaration of interest

H C D is a scientific advisor and owns shares in Integrated Nanotherapeutics. C B V is a co-founder and own shares in Integrated Nanotherapeutics. These activities do not pertain to the content covered in this review.

Funding

H C D is supported by a Juvenile Diabetes Research Foundation Postdoctoral Fellowship and Canadian Diabetes Association Postdoctoral Fellowship. C B V was supported by an investigator award from BC Children's Hospital and the Irving K. Barber Chair in Diabetes Research, and research is supported by the Canadian Institutes of Health Research (MOP-123338; MOP-130518; MOP-14682), Canadian Diabetes Association (OG-3-11-3413-CV), Stem Cell Network, and Juvenile Diabetes Research Foundation (3-SRA-2014-39-Q-R).

Author contribution statement

H C D and C B V wrote and edited the manuscript.

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Received in final form 30 November 2017

Accepted 6 December 2017