

REVIEW

The roles of vitamin D and cathelicidin in type 1 diabetes susceptibility

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Abstract

Type 1 diabetes has an increasingly greater incidence and prevalence with no cure available. Vitamin D supplementation is well documented to reduce the risk of developing type 1 diabetes. Being involved in the modulation of cathelicidin expression, the question whether cathelicidin may be one of the underlying cause arises. Cathelicidin has been implicated in both the development and the protection against type 1 diabetes by mediating the interplay between the gut microbiome, the immune system and β cell function. While its potential on type 1 diabetes treatment seems high, the understanding of its effects is still limited. This review aims to contribute to a more comprehensive understanding of the potential of vitamin D and cathelicidin as adjuvants in type 1 diabetes therapy.

Key Words

- ▶ β cell protection
- ▶ cathelicidin
- ▶ diabetes
- ▶ immunomodulation
- ▶ vitamin D

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Type 1 diabetes

Diabetes mellitus refers to a group of metabolic diseases characterised by chronic hyperglycaemia due to absent insulin secretion, insulin action or both (1). Diabetes is classically divided in type 1 and type 2 diabetes (2) and of all individuals diagnosed with this disease, type 1 diabetes represents up to 10%, of which 80–90% are children or adolescents (3). Type 1 diabetes is commonly referred to as autoimmune diabetes and results from the autoimmune destruction of pancreatic β cells. On the other hand, type 2 diabetes, which accounts for the remaining 90% of cases, affects mostly adults, although its incidence in youth is increasing due to changes in lifestyle and increased obesity (4). Differently from type 1 diabetes, type 2 diabetes is characterised by insulin resistance and defective insulin secretion, which may be accompanied by the destruction of β cells (5).

Type 1 diabetes is one of the most common endocrine diseases in children (6); recent reports indicate a yearly increase from 3 to 4% on the incidence in childhood (7).

This is most concerning in children with less than 15 years and particularly less than 5 years (8).

Type 1 diabetes is a multifactorial disease. Genetic factors associated with certain haplotypes from the HLA complex have been shown to decisively influence the susceptibility (9). Additionally, environmental factors such as the seasonal environment at birth (3), infant diet (10), viral infections (11) and the gut microbiome (12) have also been suggested to play a role on type 1 diabetes development. The heterogeneity and variation in the pathogenic process and phenotypic characteristics makes it difficult to diagnose and to treat the disease at an early stage (13).

Pathophysiology

Type 1 diabetes is a chronic disease resulting from the autoimmune destruction of the insulin-producing β cells in the pancreas. Not all cases of type 1 diabetes are autoimmune mediated. For around 10–30% of patients,

the cause is idiopathic and the specific pathogenesis is unclear (14).

The triggering event that leads to the autoimmune elimination of β cells is still unknown but may be related to a failure in the elimination of self-reactive T cells, in the thymus. This leads to the escape of T cell populations – autoreactive against β cell proteins such as insulin, glutamic acid decarboxylase (GAD) and protein tyrosine phosphatase IA-2 – to the periphery (15, 16, 17). Upon encounter with the self-antigens, T cells undergo activation and expansion, releasing proinflammatory cytokines. This promotes pancreatic infiltration of more T cells, macrophages, B lymphocytes and plasma cells, with resultant autoimmune destruction of the insulin-secreting β cells (18). The symptoms are observed only when around two-thirds of the β cell mass is lost (19). During the pre-symptomatic stage, markers of autoimmunity, presence of islet autoantibodies in circulation, as well as dysregulation of blood glucose start to arise due to the loss of β cells. With the continuous decline in β cells, the symptomatic stage is reached and signs of diabetes, polyuria, polydipsia, weight loss, fatigue, diabetic ketoacidosis (DKA) are identified (20). The rate of progression to the symptomatic stage can vary from months to decades, in both children and adults (13).

Type 1 diabetes management strategies

Given the absence or reduced insulin secretion, an obvious strategy for type 1 diabetes management involves its exogenous administration (21). Since the discovery of insulin in 1922, much advances in health care has been achieved, such that the previously terminal disease is now treatable. Nowadays, the availability of new insulin analogues, with varying duration of activity, allows for a multiple-dose insulin therapy that better resembles the physiologic insulin release (22). The combination of the rigorous monitoring of blood glucose levels with the multifunctional insulin therapy enables the glycaemic control, and to prevent, or delay, the complications of type 1 diabetes (23).

Despite these advances, type 1 diabetes is still associated with a high medical, psychological and financial burden. Hypoglycaemia and ketoacidosis remain life-threatening complications, as well as an increased risk of co-morbidities (cardiovascular disease, retinopathy and nephropathy) and premature death (22, 24).

Efforts to manage the disease are not able to reach a cure but only a control of the symptoms. Given the heterogeneity of type 1 diabetes, treatment should

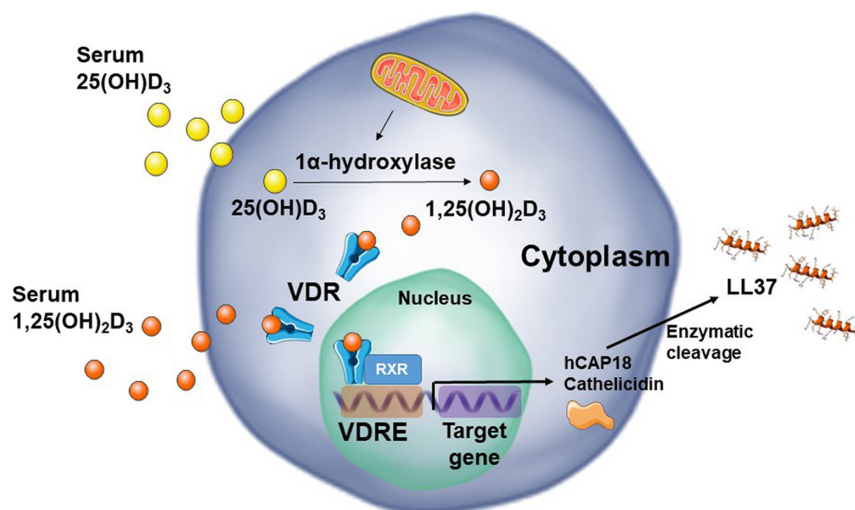
also be looked as a multivalent strategy. Alternative strategies have been explored, including the use of immunosuppressant therapies, which leave the patients immunocompromised and susceptible to infections; the use of antigenic tolerance therapies, the protection of β cells and selective stimulation of their proliferation or reprogramming of non- β cells into functional β cells (19). Although islets have limited regenerative capacity it has been found that, within islets, α cells and δ cells can undergo transdifferentiation to functional β cells or β -like cells (25, 26). Pancreatic β cells replicate at a high rate during the foetal and neonatal stages, a process that rapidly declines with age (27). Inducing β cells to undergo mitosis using harmine and 5-iodotubercidin, inhibitors of the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), has shown promising to the recovery of β cell mass (28, 29). However, the limited potency and lack of β cell specificity, with undesirable off-target effects, limit their applicability. A drug able to stimulate β cell regeneration while simultaneously shifting the proinflammatory autoimmune islet milieu to an anti-inflammatory one, to prevent future insulinitis, would be the ideal candidate for type 1 diabetes cure (19).

Vitamin D and the antimicrobial peptide cathelicidin have been proposed as promising candidates for this endeavour. On one hand, both pre-clinical and clinical research demonstrated vitamin D has a well-established role in the protection from type 1 diabetes development. On the other hand, although cathelicidin expression is directly induced by vitamin D, the mechanistic effects of cathelicidin in type 1 diabetes susceptibility and therapy are still poorly understood.

Vitamin D

Vitamin D₃ (cholecalciferol, hereafter mentioned as D₃) is a liposoluble molecule precursor of human steroid hormones, which can be obtained through diet or exposure to UV-B sunlight. After production, D₃ is partially stored in adipose tissues in a few hours, while other part is converted to 25-hydroxyvitamin D₃ (25(OH)D₃) by the liver. 25(OH)D₃ is further hydroxylated into the active form 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) in the kidneys (30), which then acts to maintain serum calcium levels, although its activities go beyond calcium homeostasis and bone metabolism (31).

The vitamin D receptor (VDR) has been identified in practically all immune cell types (32). After intracellular enzymatic activation of vitamin D and subsequent VDR

**Figure 1**

Classic vitamin D signalling pathway. Serum 25(OH) $_2$ D $_3$ is converted to 1,25(OH) $_2$ D $_3$ by mitochondrial 1 α -hydroxylase, initiating the signalling cascade by binding to VDR, which dimerizes with RXR, and binds to specific DNA sequences, VDREs, regulating the transcription of several genes. 1,25(OH) $_2$ D $_3$ -VDR-RXR complex then synthesizes cathelicidin as an inactive pro-peptide (hCAP18) which is cleaved upon release of the active LL-37 by proteases.

binding, dimerisation with retinoid X receptor (RXR) induces gene transcription at specific DNA sequences, termed vitamin D response elements (VDRE) (Fig. 1). 1,25(OH) $_2$ D $_3$, a potent pleiotropic hormone, acts as a molecular switch targeting hundreds of known human genes across a wide variety of tissues, including the human cathelicidin which will be later discussed (33). Many cell types are able to co-express VDR and the 25(OH) $_2$ D $_3$ -activating enzyme, 1 α -hydroxylase, including macrophages, dendritic cells and β cells, enabling the intracrine metabolism and action of 1,25(OH) $_2$ D $_3$ generated from 25(OH) $_2$ D $_3$ (34, 35).

Immunomodulatory roles of vitamin D in type 1 diabetes

Vitamin D has the ability to downregulate adaptive immunity and induce immunological tolerance and anti-inflammatory effects, being significant in the context of autoimmune diseases. The immunomodulatory effects of 1,25(OH) $_2$ D $_3$ with particular relevance for type 1 diabetes are presented in Fig. 2. 1,25(OH) $_2$ D $_3$ exerts immunomodulatory effects in the interplay between dendritic cells (DCs), macrophages, T CD8+ (cytotoxic), T CD4+ (helper) and B lymphocytes.

In the context of type 1 diabetes, T CD8+ cells bearing a self-reactive T cell receptor can recognise self-antigens present on β cell surface, on MHC I, inducing apoptosis of the β cell through perforin or Fas/Fas ligand interactions. These self-antigens can also be picked up by resident or recruited DCs or macrophages which present these antigens to T CD4+ cells. In turn, these T cells may then directly kill the nearby β cells, initiate the immune response producing soluble mediators that induce β cell

death or recruit other immune cells to the pancreas which further damage the β cells (36). 1,25(OH) $_2$ D $_3$ inhibits the differentiation, activation and maturation of DCs as well as the expression of MHC II (37, 38, 39), decreasing autoantigen presentation and, thus, preventing the first step in the initiation of an immune response. Additional effects on DCs include downregulation of proinflammatory IL-12 and TNF α , and simultaneous upregulation of anti-inflammatory IL-10, TGF β and stimulation of immunosuppressive T-reg cells (40, 41, 42). 1,25(OH) $_2$ D $_3$ can also inhibit the differentiation of monocytes into macrophages and the activation of T CD8+ cells (43, 44).

The presentation of the antigens by DCs and macrophages to T CD4+, leads to their differentiation into T helper (Th) 1 and Th17, which further stimulate IL-12 and IFN γ production, consequently improving T CD8+ and macrophage attack to β cells. By decreasing the IL-12 secretion required for T CD4+ activation and stimulating the release of IL-10, 1,25(OH) $_2$ D $_3$ inhibits the proliferation, activation and differentiation of TCD4+ cells and enhances the prevalence of T-regs. Furthermore, the activation of T-regs promotes the downregulation of proinflammatory Th1 cytokines and favours anti-inflammatory Th2 cytokines (45), diminishing type 1 diabetes progression. The presence of 1,25(OH) $_2$ D $_3$ was reported to decrease IL-22-expressing CD4+ T cells and IFN γ accompanied by an increase in IL-4 levels (46). Likewise, 1,25(OH) $_2$ D $_3$ effects on macrophages include the shift from a proinflammatory profile to an anti-inflammatory one, by downregulating proinflammatory mediators, such as IL-1 α , IL-1 β , IL-6 and TNF- α (47, 48), and increasing IL-10 production (49).

Following the initiation of the autoimmune process in type 1 diabetes, a further humoral reaction is triggered by B

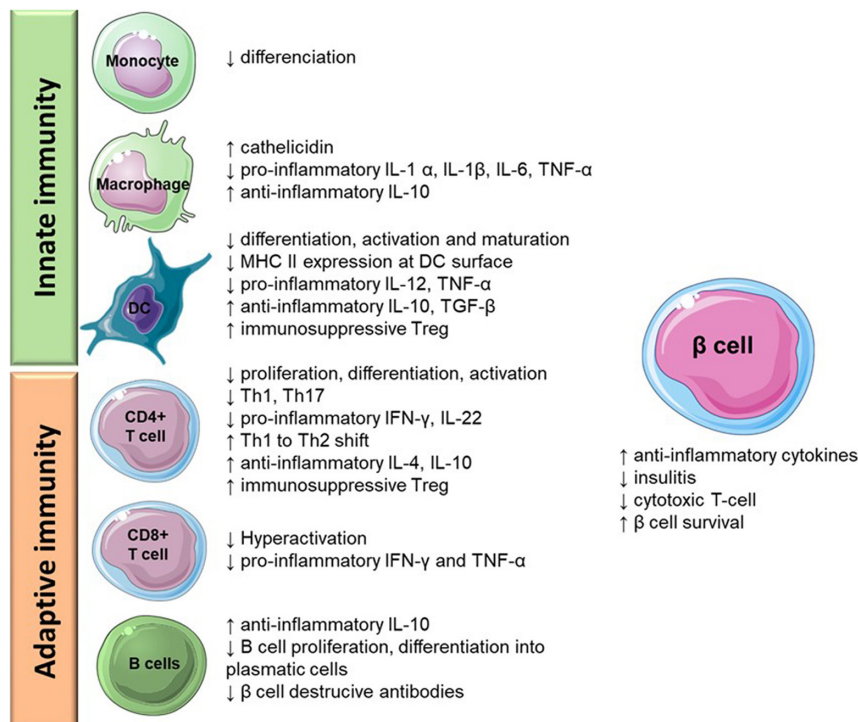


Figure 2
Immunomodulatory actions of 1,25(OH)₂D₃ in innate and adaptive immunity.

lymphocytes that differentiate into plasma cells and secrete antibodies that contribute to β cell destruction. 1,25(OH)₂D₃ inhibits B lymphocyte differentiation and proliferation (50, 51), consequently reducing destructive antibodies. 1,25(OH)₂D₃ enhances IL-10 expression by activated B lymphocytes by more than threefold, mostly by recruiting the VDR to the promoter of IL-10 (52). Drozdenko *et al.* further showed that 1,25(OH)₂D₃-primed B cells display an impaired capacity to activate T cells (53). Overall, vitamin D exhibits a protective effect on β cells survival, which ultimately delays type 1 diabetes progression.

Vitamin D role in diabetes prevention and treatment from pre-clinical to clinical evidence

The NOD mice model opened doors to several *in vivo* evidences on vitamin D potential as an adjuvant for both prevention and treatment of type 1 diabetes. Mathieu *et al.* long ago reported that treatment with 1,25(OH)₂D₃ not only reduced insulinitis incidence, but more importantly, also reduced the cumulative incidence of diabetes (54). Short treatment of NOD mice with Ro 26-2198, a 1,25(OH)₂D₃ analogue, inhibited IL-12 production, blocked pancreatic infiltration of Th1 cells and arrested the progression of type 1 diabetes (55). Vitamin D deficiency plays a determining role by increasing the incidence of diabetes in female NOD mice from 46 to 88% and from

0 to 44% in male mice, when compared to vitamin D sufficient animals (56). Additionally, oral administration of 1,25(OH)₂D₃ significantly delayed disease onset, although also causing a significant rise in calcium serum levels. Furthermore, mice lacking a functional VDR exhibited impaired insulin secretory capacity compared to controls (57). In another study, vitamin D privation increased type 1 diabetes incidence and glucose intolerance (58), a precocious disease manifestation, suggesting a role of vitamin D in slowing down the development of type 1 diabetes. Concordantly, the lifelong oral administration of high doses of 1,25(OH)₂D₃ safely prevented diabetes in NOD mice, both female and male, resulting in a decrease in cytotoxic T cells and an increase of T-regs, reducing severe insulinitis and improving β cell function (59).

Compelling evidence from pre-clinical data encouraged the search for a clinical proof of concept. Serum 25(OH)D₃ levels depend on many factors such as region, gender, season and age, normally in the 30–68 ng/mL range, whereas concentrations <20 ng/L are considered deficient (60). Several studies have reported significantly lower levels of 25(OH)D₃ in type 1 diabetes subjects compared to healthy controls (61, 62, 63), even in an environment of abundant sunlight (64). In the last 20 years, many clinical studies have been performed on the use of vitamin D and derivatives as supplements to insulin in type 1 diabetes patients.

In a preventive context, D₃ supplementation in children with ≥ 2000 IU daily has shown to reduce by 80% the risk of developing type 1 diabetes (65). Moreover, a meta-analysis of observational studies showed that in at least five reports vitamin D intake during early childhood is significantly associated with a reduced risk of type 1 diabetes (66). D₃ supplementation, with doses ranging from 2000 IU/day to 4000 IU/day, has been shown to decrease HbA1c (glycated haemoglobin) levels, while increasing T-regs and C-peptide levels (a marker of insulin secretion) (67, 68, 69). In an attempt to avoid adverse effects, 0.25 μg of 1,25(OH)₂D₃ administered on alternate days for 1 year along with regular insulin therapy showed a modest effect on residual pancreatic β cell function and reduced the required insulin dose, although temporarily (70). Nevertheless, the same regimen has also been reported by others to have no beneficial effects of β cell function and insulin requirements (71, 72).

New analogues, with structural modifications and non-calcemic, are emerging and may help to overcome the issue of dosage limits. One of them, alfacalcidol (1 α -hydroxycholecalciferol) has been shown to protect β cell function at a dose of 0.25 μg twice daily, as observed by a raise in C-peptide levels in children (73). Long-term treatment using this analogue appears to be safe and is likely to reduce the risk of hypercalcemia, as compared to 1,25(OH)₂D₃ administration. Other analogues tested in humans have shown higher efficacy, namely BXL-219 (formally Ro 26-2198) (74) and TX527 (75). Nevertheless, high serum calcium may be avoided not only by using analogues. Sixth-month supplementation with the precursor 25(OH)D₃ in young patients, with increasing dosage, was reported to safely restore and maintain 25(OH)D₃ levels up to 1 year after treatment; peripheral blood mononuclear cell reactivity against β cell autoantigens was reduced with no significant decrease of β cell function (76). The ability of immune cells to locally convert 25(OH)D₃ into 1,25(OH)₂D₃, may support the use of this vitamin D metabolite over its analogues.

Altogether, vitamin D deficiency significantly increases the susceptibility to type 1 diabetes and its supplementation may improve glycaemic control. Vitamin D is an inexpensive and readily available candidate for autoimmune therapy. In addition to 1,25(OH)₂D₃-induced downregulation of cytokine/chemokine production, stimulation of cathelicidin gene expression represents another very important pathway by which vitamin D regulates the innate immune response. Patients with 25(OH)D₃ blood levels under 20 ng/mL

may be unable to fully express cathelicidin (77), which could lead to increased susceptibility to type 1 diabetes.

Cathelicidin

Cathelicidins are small cationic peptides, with a size varying from 12 to 80 amino acid residues (78), expressed in many different species of mammals, the best-characterised are the human (LL-37), mouse and rat cathelicidin-related antimicrobial peptide (mCRAMP and rCRAMP). These naturally occurring antimicrobial peptides (AMPs) display a plethora of activities and a fundamental role in the innate immune system (79, 80).

AMPs are evolutionarily conserved molecules of the innate immune system present in all complex organisms (81). As many other AMPs, cathelicidin has a modest antimicrobial activity against a broad range of pathogens including fungi, bacteria, enveloped viruses and protozoa (82). AMPs have a small size, 37 residues in the case of LL-37, being generally cationic due to the excess of lysine and arginine residues. This feature combined with the presence of around 50% hydrophobic residues favours interaction with the negatively charged membrane of bacteria which lead to its disruption (83).

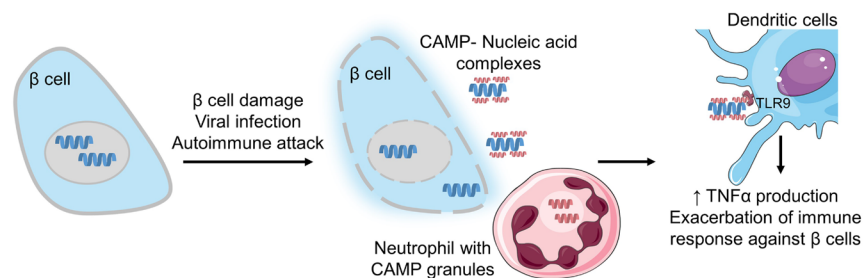
In humans, cathelicidin is synthesized as a preproprotein, the human cathelicidin antimicrobial protein (hCAP18), which must be cleaved by serine proteases upon its exocytosis to become the active LL-37 (Fig. 1) (84).

Cathelicidin has a broad range of activities, with a pleiotropic role in innate immune responses and inflammation. It has an important immunomodulatory activity (79), with anti and proinflammatory effects in different cells, according with the inflammatory environment (85), being chemoattractant for leucocytes (86), and also being involved in tissue healing, revascularisation, cell proliferation and differentiation (80).

Vitamin D and cathelicidin partnership in type 1 diabetes

The expression of LL-37 is directly induced by vitamin D (87, 88) (Fig. 1), suggesting that the beneficial effect from vitamin D supplementation may be connected to a restoration of cathelicidin levels and raising the question whether a deficiency in LL-37 may be one of the underlying causes for type 1 diabetes susceptibility.

Positive correlation between circulating cathelicidin and 25(OH)D levels in healthy adults has been

**Figure 3**

CAMP involvement in type 1 diabetes development in NOD mice. After a triggering event, which induces β cell damage, the release of self-nucleic acids may occur. The presence of neutrophils in the pancreatic tissue, which releases the CAMP, may result in immune complexes which activate dendritic cells via the toll-like receptor (TLR9) and enhancing the production of proinflammatory cytokines such as TNF α which exacerbate the immune response against β cells.

demonstrated (89). Exogenous administration of cathelicidin to increase serum levels is hampered by toxicity risks, however, vitamin D supplementation may be used as a strategy to indirectly increase LL37 serum levels.

To the best of our knowledge, no information is available on the clinical interplay between vitamin D, LL-37 concentration and disease severity in patients with type 1 diabetes. This topic has been relatively unexplored so far in pre-clinical studies probably because the pathway by which 1,25(OH) $_2$ D $_3$ regulates the expression of cathelicidin gene is restricted to humans and other non-human primates. Since mice and other experimental animal models lack the VDRE in their cathelicidin gene promoters (90, 91), *in vivo* studies on this topic using mice would have limited significance.

Nevertheless, Zhou *et al.* (92) showed that 25(OH)D $_3$ attenuates periodontitis by promoting the expression of cathelicidin in mice with type 2 diabetes, however, this may have resulted from an indirect immunomodulatory effect of vitamin D on intracellular signalling.

Recently, a transgenic mice that carries a genomic DNA fragment encompassing the entire human CAMP gene was generated (93). In this study, topically applied 1,25(OH) $_2$ D $_3$ induced CAMP expression and boosted *Staphylococcus aureus* killing. In the future, humanised mice may help to elucidate the biologic role of cathelicidin and vitamin D in type 1 diabetes.

Controversial role of cathelicidin role in type 1 diabetes development vs treatment

The intriguing role of cathelicidin in the pancreas was uncovered only recently. Diana *et al.* (94) hypothesised that in NOD mice, mCRAMP is involved in the initial stages of type 1 diabetes development. The authors demonstrated that, in young female NOD mice, the infiltration of innate immune cells, including neutrophils able to secrete cathelicidins, occurs as early as 2 weeks of age. If β cell damage has occurred, self-DNA can be present extracellularly and through electrostatic interactions with cathelicidin may

form immune complexes able to activate macrophages and DCs via the toll-like receptor 9 (TLR9). This exacerbates the immune response by stimulating the release of IFN- α which can be deleterious, as observed in other autoimmune conditions (95, 96) and possibly initiating the inflammatory milieu of type 1 diabetes (94) (Fig. 3).

However, in different reports, the serum concentration of LL-37 has been shown to be reduced in patients with type 1 diabetes, as compared to that of healthy subjects (97, 98) and although not significantly different, this finding suggests another role of cathelicidin in type 1 diabetes. Later, it was found that mCRAMP is also secreted by islets of various strains of mice, the ones of NOD mice secreting the lowest amounts (99). In healthy α and β cells, from both human and mice islets, cathelicidin is expressed and secreted constitutively, being scarce in female NOD mice, which are most susceptible to type 1 diabetes development (99). The role of LL-37 appeared even more relevant since the intraperitoneal administration of mCRAMP led to a change in the pancreatic immune infiltrate, increasing the relative abundance of M2 macrophages and T-regs. Given that an immunosuppressive phenotype is inversely associated with type 1 diabetes risk, cathelicidin treatment resulted in a lower incidence of autoimmune diabetes in NOD mice (99). Concordantly, diabetes-prone rats which were fed with a protective-diet that delayed the onset of type 1 diabetes, showed an increased expression of cathelicidin in the epithelial lining of the small intestine and an increase in the relative abundance of M2-macrophages (100). Sun *et al.* (99) also demonstrated that CRAMP expression in the islets was inducible and dependent on short-chain fatty acids (SCFA), in particular butyrate, which in mammals are produced by bacteria of the gut during the fermentation of dietary fibres, thus establishing cathelicidin as a link between the gut microbiome and immune-regulation of the pancreatic immune environment (Fig. 4).

The mechanisms by which cathelicidins are produced in the islets and modulate type 1 diabetes development were further unveiled by Sun *et al.* (101). In this study, the authors showed that rCRAMP is constitutively expressed

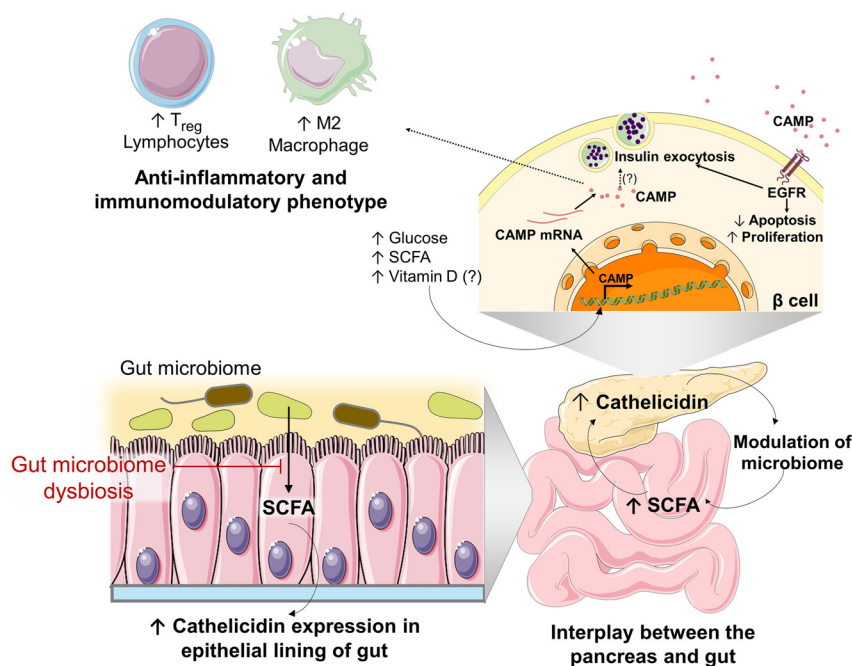


Figure 4

Model summarising the role of cathelicidin in the interplay between the pancreas and the gut. Short-chain fatty acids (SCFA) are produced by the gut microbiome during alimentary fibre fermentation, stimulating the expression of cathelicidin in the epithelial lining of the gut. SCFA stimulates the expression of cathelicidin by pancreatic islets, which reciprocally control and modulate the bacterial growth in the gut, preventing gut microbiome dysbiosis. Cathelicidin expression in the β cell is also induced in the presence of high glucose concentration and may also be induced by vitamin D, as seen in other cell types. Extracellular cathelicidin stimulates insulin granule exocytosis via the epidermal growth factor receptor (EGFR). It is not completely elucidated if intracellular cathelicidin can also induce insulin release directly. Cathelicidin expression also induces the prevalence of regulatory T cells (T-regs) and M2 macrophages which are associated with a lower risk of type 1 diabetes development.

in the INS-1 832/13 insulinoma cell line (β cell model), promoting cell viability and growth via the EGFR, by increasing the expression of anti-apoptotic proteins and by modulating the cleavage of caspase, overall protecting β cells from apoptosis. Furthermore, pre-treatment with an EGFR inhibitor only partially blocked the cathelicidin stimulatory effect on cell viability, suggesting that the peptide may also act on β cells through other receptors (101). Importantly, it was observed that cathelicidin promoted the glucose-stimulated insulin release in isolated rat and mouse islets and improved the glucose tolerance in NOD mice (101) (Fig. 4).

In a report by Pound *et al.* (102), the expression of cathelicidin was also detected in β cells of rats, mice and human islets, but not on α cells, contrary to what was previously stated (99). In bio-breeding diabetes-prone (BBdp) rats, downregulation of rCRAMP mRNA of approximately 60%, was observed before the onset of insulinitis, again suggesting a connection of cathelicidin with diabetes susceptibility (102). The gluoregulatory effect was further demonstrated in isolated islets in which cathelicidin treatment promoted a significant increase in insulin or glucagon secretion, in a glucose-dependent manner. Reciprocally, cathelicidin expression was also induced after exposure to higher glucose concentration (16.7 mM) (Fig. 4), which suggests a role of cathelicidin in islet paracrine signalling that enhances islet function and gluoregulation (102). Notably in *in vivo* studies, the treatment of BBdp rats with rCRAMP showed short-term (after a single administration) insulin release profile

comparable to that of BB control rats and long term (1-week, daily administrations) enhancing β cell neogenesis from pancreatic duct cells (102). Since type 1 diabetes patients have a significant loss of β cell mass, the ability to enhance β cell regeneration and function could be therapeutically valuable (102). Additionally, cathelicidin expression was found to be downregulated in the gut of BBdp rats, which showed a clear shift in abundance of populations of gut microbiota, effects that were partially normalized by treatment with rCRAMP (102) (Fig. 4).

Further connection between cathelicidin and the microbiome was demonstrated by Ahuja *et al.* (103) in mice knocked-out for a calcium channel (Orai1), dampening the release of AMPs to the gut by the acinar cells from the exocrine pancreas. This allows bacteria outgrow and dysbiosis, intestinal inflammation, systemic infection and death, effects that can be prevented by mCRAMP supplementation, restoring the phenotype of Orai1-deficient mice (103). These studies reveal once more a close relationship between the pancreas and the intestine. Disturbing this balance can cause predisposition to pancreatic and intestinal diseases (104).

Deng *et al.* (105) showed that cathelicidin is also implicated in the protection against acute pancreatitis (AP). Immune cell infiltration, release of inflammatory mediators and apoptosis of pancreas acinar cells are characteristic of AP, which can evolve to diabetes depending on the severity and extent of pancreatic necrosis (106). In mice in which the cathelicidin gene was knocked-out, the severity of drug-induced AP was higher, with greater

neutrophil infiltration, a more pronounced acinar cell injury and an increased production of proinflammatory cytokines, relative to the wild-type mice, showing that the anti-inflammatory effects of cathelicidin protect mice from AP (105).

With the exception of a recent report by Stenwall *et al.* (107), in recent years this topic was not further explored. In this study, the expression of several AMPs in the exocrine and endocrine pancreas of healthy and diabetic patients was evaluated and the presence of cathelicidin in the islets was detected using immunohistochemistry. These authors also reported a generally lower level of antimicrobial peptides in the pancreas of a type 1 diabetes donor (107).

Overall, these reports indicate that the absence of cathelicidin is associated with a higher risk of pancreatic inflammation and type 1 diabetes. Consistently, its administration seems to improve β cell function, normalise microbiome imbalances and reduce the inflammatory response. Therefore, its potential applicability in type 1 diabetes therapy is promising, although further studies are required.

Concluding remarks

Vitamin D as well as cathelicidin have been implicated in both type 1 diabetes susceptibility and protection. Although the role of vitamin D has been subject to a more extensive research, the role of cathelicidin and the mechanistic effects by which its expression from β cells may help prevent type 1 diabetes still need further exploration. The main results revised in this state of the art suggest that cathelicidin may mediate the interplay between the gut microbiome, the immune system, β cell function and type 1 diabetes development and that the serum vitamin D may modulate those activities. Further studies are required to understand whether cathelicidin may be therapeutically valuable in type 1 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

C C and A M drafted the manuscript. B S and F M G designed the topic of research and topics to discuss. All authors critically revised and approved the manuscript.

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