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The role of Interleukin-32 in autoimmunity

Rafael de Albuquerque | Elina Komsi | Inna Starskaia | Ubaid Ullah | Riitta Lahesmaa

Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland

Correspondence
Riitta Lahesmaa and Ubaid Ullah, Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland.
Emails: rilahes@utu.fi (R. L.); ubaull@utu.fi (U. U.)

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Abstract

Interleukin-32 (IL-32) is a pro-inflammatory cytokine that induces other cytokines involved in inflammation, including tumour necrosis factor (TNF)-α, IL-6 and IL-1β. Recent evidence suggests that IL-32 has a crucial role in host defence against pathogens, as well as in the pathogenesis of chronic inflammation. Abnormal IL-32 expression has been linked to several autoimmune diseases, such as rheumatoid arthritis and inflammatory bowel diseases, and a recent study suggested the importance of IL-32 in the pathogenesis of type 1 diabetes. However, despite accumulating evidence, many molecular characteristics of this cytokine, including the secretory route and the receptor for IL-32, remain largely unknown. In addition, the IL-32 gene is found in higher mammals but not in rodents. In this review, we outline the current knowledge of IL-32 biological functions, properties, and its role in autoimmune diseases. We particularly highlight the role of IL-32 in rheumatoid arthritis and type 1 diabetes.

1 | INTRODUCTION

Interleukin 32 (IL-32) was first identified in 1992 as a novel human gene encoding a 27-KD protein expressed in activated natural killer (NK) cells and T cells in humans and was named NK4.[1] In 2005, Kim et al found that the product of NK4 possesses the characteristics of a pro-inflammatory cytokine, and accordingly, the name was changed to IL-32.[2] A growing body of evidence implicates IL-32 in health and disease. Its roles in host responses to pathogens[3,4] allergy and asthma,[5] cancer[6] and cardiovascular diseases[7] have been reviewed recently. The objective of this review is to discuss the role of IL-32 in autoimmune diseases and, in particular, rheumatoid arthritis (RA) and type 1 diabetes (T1D).

2 | GENE AND ISOFORMS

The *IL32* gene has at least eight exons that reside on human chromosome 16p13.3.[2] Interestingly, the gene is encoded in higher mammals but not in rodents.[8] As of May 2020, the Ensembl database (ensembl.org) contains 35 splice variant transcripts of the gene, of which 30 potentially encode proteins. The longest IL-32γ isoform is identical to the original NK4 transcript, and alternative splicing yields nine experimentally validated isoforms: IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32 and IL-32sm.[2,8-10] The most-studied isoforms are IL-32α, IL-32β, IL-32γ and IL-32δ, with gamma being the longest and the most-studied isoform. IL-32α, IL-32β and IL-32γ are structurally similar to each other, and IL-32η and IL-32θ are similar to each other. The

Rafael de Albuquerque, Elina Komsi, Inna Starskaia and Ubaid Ullah contributed equally to this work

smallest isoform IL-32sm is very short and only contains the last two exons.[6] All IL-32 isoforms contain a tripeptide RGD motif, which is present in extracellular matrix proteins (e.g. fibronectin) and helps in cell adhesion and movement. Through these motifs, the secreted forms of IL-32 may bind to integrins as the integrins interact with RGD motif-containing proteins. Integrins, therefore, may serve as receptors for IL-32. Although IL-32 is functionally similar to a cytokine, it has no sequence or structural similarity to any known cytokine.[8,11]

3 | IL-32 EXPRESSION IN HEALTH AND DISEASE

After the identification of IL-32 expression in NK cells, several other immune cells including T cells have been shown to express IL-32 (Table 1). High levels of expression in T and NK cells were confirmed in a single-cell RNA-seq study of peripheral blood mononuclear cells,[12] suggesting that IL-32 has a critical function in T and NK cells and a significant role in T and NK cell-mediated autoimmune pathologies. In a database (https://www.genenetwork.nl) of mRNA expression from 31 499 public RNA-seq samples, IL-32 was expressed in a wide range of tissues with the highest expression in spleen, followed by liver, thymus and muscle (https://www.genenetwork.nl). Average expression was detected in pancreas and blood along with other tissues, but expression in prostate, ovary, breast and brain was low. Highest expression in the spleen was also found in NIH Genotype-Tissue Expression (GTEx) project. Among peripheral blood leukocytes, the highest expression was found in memory Treg cells (defined as $CD3^+$ $CD4^+$ $CD45RA^-$ $CD25^{high}$ $CD127^{low}$), followed by effector CD4 cells, CD8 cells, and NK cells, but no expression was detected in non-stimulated B cells and monocytes sorted from freshly obtained PBMC (https://dice-database.org/) (Figure 1). However, IL-32 expression obtained through these databases requires further confirmations.

IL-32 expression altered in different cancers[6] and in viral, bacterial, and protozoan infections[3,4,13] Furthermore, IL-32 expression appears to be induced in several autoimmune diseases, which will be covered later. Different stimuli induce IL-32 expression in various cell types.[8] IL-32 was induced by IL-2 in NK cells and by mitogens in T cells.[1] IL-18 induced IL-32 in a macrophage cell line.[2] TNF-α induced IL-32 in synovial fibroblasts[14] and monocytes.[15] Interestingly, while IL-32 was not detected in non-stimulated sorted monocytes

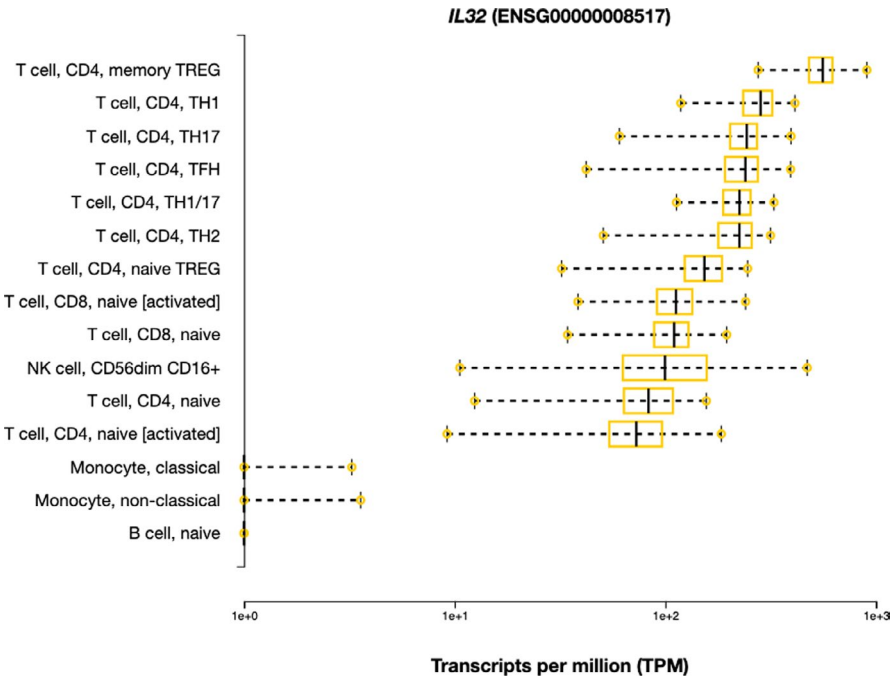

FIGURE 1 Expression of IL-32 in ex vivo immune cells from 91 healthy donors (https://dice-database.org/). The cell types have identified using following markers. Classical monocytes: $CD3^-CD14^{high}$ $CD16^-$ $CD56^-$. Non-classical monocytes: $CD3^-CD14^-$ $CD16^+$ $CD56^-$. NK cells: $CD3^-$ $CD16^+$ $CD56^{dim}$. Naïve B cells: $CD3^-CD14^-$ $CD19^+$ $CD20^+$ $CD24^{dim}$ $CD38^{dim}$ $CD27^-$ IgM^+ IgD^+ IgG^-. Naïve $CD4^+$ T cells: $CD3^+$ $CD4^+$ $CD45RA^+$ $CCR7^+$. Naïve $CD8^+$ T cells: $CD3^+$ $CD8a^+$ $CD45RA^+$ $CCR7^+$. Naïve Treg cells: $CD3^+$ $CD4^+$ $CD25^{high}$ $CD45RA^+$ $CCR127^{low}$. Memory Treg cells: $CD3^+$ $CD4^+$ $CD25^{high}$ $CD45RA^-$ $CCR127^{low}$. TH1 cells: $CD3^+$ $CD4^+$ $CD25^{low}$ $CD45RA^-$ $CCR127^{high}$ $CXCR3^+$ $CCR4^-$ $CCR6^-$. TH1/17 cells: $CD3^+$ $CD4^+$ $CD25^{low}$ $CD45RA^-$ $CCR127^{high}$ $CXCR3^+$ $CCR4^-$ $CCR6^+$. TH17 cells: $CD3^+$ $CD4^+$ $CD25^{low}$ $CD45RA^-$ $CCR127^{high}$ $CXCR3^-$ $CCR4^+$ $CCR6^+$. TH2 cells: $CD3^+$ $CD4^+$ $CD25^{low}$ $CD45RA^-$ $CCR127^{high}$ $CXCR3^-$ $CCR4^+$ $CCR6^-$. TFH cells: $CD3^+$ $CD4^+$ $CD25^-$ $CD45RA^-$ $CXCR5^+$

TABLE 1 Different immune cell types expressing IL-32 after stimulation

Cell type	Treatment	Isoforms
NK cell	IL-2,[1] IL-12 and IL-18[2]	no specification,[1] IL-32α[2]
CD3 + T cell	anti-CD3, PMA/Ionomycine[9]	IL-32β, δ, ε, ζ [9]
CD4 + T cell	Con A, anti-CD3 and anti-CD28, IL-12, IL-18, IL-23 and TNFα[46]	IL-32 α, β, γ, δ [46]
B cell	Concanavalin A, anti-CD3 and anti-CD28, co-culture with activated CD3 + T cells[46]	IL-32 α, β, γ, δ [46]
Monocyte	Concanavalin A, anti-CD3 and anti-CD28, co-culture with activated CD3 + T cells,[46] TNFα, IL-1β, IFNγ + TNFα[15]	IL-32 α, β, γ, δ,[46] no specification[6]
PBMC	Concanavalin A, LPS,[2] no stimulation[12,37]	IL-32α,[2] IL-32 α, β, γ,[12]IL-32α[37]

(https://dice-database.org/), it could be induced in these cells upon TNF-α treatment. This underlines that IL-32 expression can vary significantly in response to environmental stimuli. Long-term TNF-α treatment induced hypomethylation of the *IL32* promoter region in HEK293 cells, leading to sustained upregulation of IL-32 even after withdrawal of TNF-α.[16]

4 | SECRETORY FORMS OF IL-32

Although IL-32 is predominantly in the cytoplasm, secretory forms have been reported.[2,9,17] IL-32γ is the only isoform with a hydrophobic signal peptide in the N-terminus, typical of a secreted cytokine,[18] but the precise mechanism of its secretion remains to be elucidated. IL-32 has been detected in the supernatant of CD3-activated T cells, where it may have been released passively from the cytoplasm of apoptotic cells.[9] It is present in synovial fluid of RA patients and can be secreted by fibroblast-like synoviocytes (FLS).[19] One study showed that IL-32 could be secreted via a non-classical secretory route in a membrane inserted form.[20]

5 | RECEPTOR FOR IL-32

The specific receptor for IL-32 has not been identified. Receptors for several cytokines, for example, IL-2, IL-6, IFNγ have been identified using affinity chromatography of crude urine extract as shedding of cytokine receptors is a general phenomenon.[21] However, a similar analysis did not identify a receptor for IL-32.[22] Instead, proteinase-3 (PR3) was identified as a specific and high-affinity interactor of IL-32α.[22] PR3 is a serine protease present in membrane-bound and extracellular form in neutrophils and monocytes that cleaves several cytokines for increased activity. Limited proteolysis of IL-32α by PR3 resulted in increased cytokine activity demonstrated by enhanced IL-32-induced MIP-2 and IL-8 production in mouse Raw cells and human PBMC, respectively.[22] Furthermore, as discussed earlier, all isoforms of IL-32 contain an RGD motif through which the IL-32α isoform binds to integrins αVβ3 and αVβ6 extracellularly.[23]

6 | FUNCTIONS OF IL-32

The known functions of IL-32 include induction of pro-inflammatory cytokine production, differentiation and apoptosis. In THP-1 cells, IL-32 stimulated the production of pro-inflammatory cytokines TNF-α, IL-1β, IL-8 and IL-6 by activating nuclear factor-kappa B (NF-kB) and mitogen-activated protein kinase (MAPK) p38.[2] In monocytes, IL-32γ enhanced the nucleotide oligomerization domain (NOD)-1- and NOD-2-induced IL-1β and IL-6 production in a caspase 1-dependent manner but did not alter TLR-induced cytokine secretion.[24] Moreover, IL-32 induced the differentiation of monocytes to macrophages.[25] IL-32 was required in the NOD-ligand-dependent dendritic cells differentiation from monocytes in leprosy.[26] There are conflicting reports on the role of IL-32 in apoptosis. It inhibited the apoptosis of breast cancer cell line MCF-7 after glucose deprivation,[27] but in T cells and HeLa cells, it induced apoptosis.[9] The inhibitory effects of IL-32 on apoptosis are consistent with an analysis of IL-32-coregulated genes. Based on the co-expression of analysis of 31 499 public RNA-seq samples (https://www.genenetwork.nl), baculoviral IAP repeat-containing 3 (*BIRC3*), which has an antiapoptotic role, was the gene most coregulated with IL-32. Furthermore, TNF receptor binding was the top molecular function of *IL32* co-expressed genes besides integrin binding and other terms.

Different isoforms of IL-32 may have different functions. Overexpression of IL-32γ in human HEK293T showed a higher cytotoxic capacity than IL-32β and in particular than IL-32α that did not show any cytotoxic effect.[28] The structural similarities of IL-32β and IL-32δ isoforms suggest that they have related biological activity. Interestingly, different isoforms of IL-32 may physically interact: IL-32η has been shown

by immunoprecipitation to interact with both IL-32β and IL-32γ.[10] Furthermore, they may also regulate the expression of other isoforms as is the case for IL-32δ, which inhibits IL-10 production via modulation of IL-32β.[29] Since most IL-32 isoforms are not secreted,[2] intracellular targets of IL-32 are worth studying in T cells and FLS (e.g. by siRNA/CRISPR-mediated silencing of the gene in a cell type-specific manner).

Although IL-32 has mostly been thought of as a pro-inflammatory cytokine, it is anti-inflammatory in some cases. For instance, IL-32β isoform promoted IL-10 expression in myeloid cell lines and primary monocyte-derived dendritic cells, leading to inhibition of immune responses.[30] Anti-inflammatory effects have also been reported for the IL-32θ isoform. IL-32θ inhibited phorbol 12-myristate 13-acetate (PMA)-induced TNF-α expression by inhibiting phosphorylation of p38 MAPK, inhibitor of κB (IκB), and NF-κB in leukaemia cell lines.[31] In addition, it decreased PMA-induced IL-1β expression in THP-1 cells.[32] The closest variant of the IL-32θ isoform is IL-32ε, and both are close to IL-32β,[33] suggesting that IL-32ε may also have anti-inflammatory properties. Based on the data so far, it appears that the pro- and anti-inflammatory properties of IL-32 are isoform dependent: IL-32γ and IL-32δ, isoforms are predominantly pro-inflammatory, whereas IL-32θ is anti-inflammatory. IL-32α and IL-32β have both pro- and anti-inflammatory properties (Table 2).

7 | IL-32 IN AUTOIMMUNITY

The pro-inflammatory nature of IL-32 made it of great interest in the context of autoimmune diseases. Though most of the studies have been done in inflammatory bowel disease[34,35] and RA, IL-32 is also involved in a range of autoimmune disorders, including T1D,[12] ankylosing spondylitis[36] and granulomatosis with polyangiitis.[37] Furthermore, IL-32 is strongly expressed in peripheral blood mononuclear cells (PBMC) and in serum from patients with Grave's diseases.[38] Similarly, IL-32 expression was elevated in sera from patients with myasthenia gravis patients[39] and PBMC from patients with psoriasis.[40] Table 3 summarizes the expression of IL-32 in autoimmune diseases. Although IL-32 appears to be a player in many autoimmune diseases, here, we will summarise the progress made in understanding the role of IL-32 in two complex autoimmune diseases: RA and T1D.

7.1 | IL-32 and rheumatoid arthritis

RA is a chronic autoimmune disease that primarily affects peripheral synovial joints and leads to their impaired mobility and destruction.[41] Different cell types, including T cells, B cells, antigen-presenting cells (APC) and FLS, contribute to the disease pathogenesis.[41] Cytokines mediate cellular interactions in the joint area and promote inflammatory processes in RA development. Pro-inflammatory cytokines, such as IL-6, TNF-α, IL-17 and IL-1β, are central to the pathogenesis of RA.[42,43] As a pro-inflammatory cytokine, IL-32 was implicated in the pathophysiology of RA.[44] It was highly expressed in the synovial tissue biopsies from the patients with RA and strongly correlated with other markers of inflammation (e.g. TNF-α, IL-1β and IL-18), as well as erythrocyte sedimentation rate and severity of the disease.[44] Further, IL-32 was one of the most highly expressed genes

TABLE 2 Immune-related functions of different IL-32 isoforms

Isoforms	Functions/properties relevant to autoimmune diseases
IL-32α	Induces the expression of pro-inflammatory cytokines, for example, IL-6, IL8. CD4 + IL-32α + T cells were significantly enhanced in Graves' disease patients compared with controls.[37] IL-32α play a protective role in EAE by suppressing neuroinflammation in spinal cord.[64] IL-32α promoted osteoclast differentiation.[54]
IL-32β	Promotes IL-10 production by IL-32β expressing K562 cells upon PMA stimulation.[28,29] Induces TNF-α production by PMA-treated THP-1 cells.[2]
IL-32 γ	Induces the expression of pro-inflammatory cytokines, for example, IL-6, IL8. Promotes migration of activated T cells via CCL5 production in DCs.[65] In ankylosing spondylitis joint, it could enhance osteoblast differentiation via DKK-1 suppression.[35]
IL-32θ	IL-32θ inhibits monocyte to macrophage differentiation by attenuating PU.1 expression.[66] IL-32θ negatively regulates TNF-α production by inhibiting p38 phosphorylation in AML patients.[31]
IL-32δ	Inhibits IL-10 production via modulation of IL-32β.[28]

TABLE 3 IL-32 in autoimmune diseases

Autoimmune disease	Study sample	IL-32 expression	Mode of action/Disease association
Type 1 diabetes (T1D)	PBMC/ T cells	IL-32 mRNA levels were upregulated in children who later developed β-cell autoimmunity (n = 7) compared with controls (n = 7)[12]	Increased IL-32 expression at the early stage may indicate its importance in the disease development; potential early indicator for disease prevention and monitoring[12]
Rheumatoid arthritis (RA)	Synovial biopsies	Expression of IL-32 was higher in patients with RA (n = 29) compared to patients with osteoarthritis and healthy controls[44]; IL-32 protein expression in synovial biopsies of RA patients (n = 16) decreased upon anti-TNF treatment[48]	IL-32 and TNF interplay amplifies inflammatory processes in RA patients[48]
Inflammatory bowel disease (IBD) comprising of ulcerative colitis (UC) and Crohn's diseases (CD)	Inflamed mucosa	IL-32α expression was higher in the inflamed mucosa of IBD patients, particularly in patients with CD (UC, n = 10; CD, n = 10) compared to healthy colorectal tissues (n = 10)[34]; IL-32ε, was increased at mRNA level in the inflamed mucosa of patients with IBD (UC, n = 18; CD, n = 15) compared to non-affected areas of the colon from the patients[35]	IL-32-driven inflammatory responses including induction of TNF-α, IL-6, IL-1β may contribute to the pathogenesis of IBD[34]; The ε isoform of lL-32 has anti-inflammatory properties that may have a protective anti-inflammatory role in the IBD mucosa[35]
Myasthenia gravis (MG)	Serum	IL-32α serum level was increased in patients with MG (n = 48) compared to healthy controls (n = 35)[39]	IL-32α serum level might be associated with disease activity in MG patients[39]
Ankylosing spondylitis (AS)	Synovial fluid and tissue	IL-32γ level in the synovial fluid and IL-32 mRNA expression in synovial tissue of AS patients (n = 15) was higher than in patients with RA (n = 17) or osteoarthritis (n = 13)[36]	High level of IL-32γ in joint fluids from the patients with AS was associated with abnormal bone formation[36]
Granulomatosis with polyangiitis (GPA)	Serum/leukocyte	IL-32 level and anti-IL-32 autoantibody were increased in the serum of GPA patients (n = 9) compared with healthy controls, also IL-32γ mRNA level expression was increased in leucocytes from GPA patients[37]	IL-32 was associated with the disease severity and its level decreases upon patient's treatment[37]
Graves´ disease (GD)	Serum/PBMC	IL-32 mRNA level expression and the proportion of IL-32α positive cells increased in PBMCs of GD patients (n = 125) compared to healthy controls (n = 97); serum IL-32 level was higher in patients compared to controls[38]	IL-32 mRNA expression positively correlated with other clinical parameters (free triiodothyronine); serum IL-32 level positively correlated with serum thyrotrophin receptor antibody and could be related to the severity of GD[38]
Psoriasis	Plasma	IL-32 levels were higher in serum from patients with psoriasis (n = 19) and psoriatic arthritis (n = 11) compared to healthy controls (n = 22)[40]	Elevated serum level of IL-32 could be a marker of inflammatory processes in the patients with psoriasis[40]

in in vitro cultured patient-derived FLS from RA patients but not from osteoarthritis patients, suggesting an important role of IL-32 in the pathogenesis of RA.[45] Various inflammatory factors, including TNF-α, IL-1β, IFN-y and pathogen-associated molecular patterns induced IL-32 expression by FLS.[46]

The mechanism of IL-32 action in RA is not fully understood; however, it may contribute to disease development

by enhancing other pro-inflammatory cytokines involved in the RA development. Recombinant IL-32 α and β isoforms induced TNF-α expression in both mouse macrophage Raw cell line and human PMA-treated monocyte THP-1 cells,[2] and conversely, TNF-α also induced IL-32 expression in T cells, monocyte-derived dendritic cells, and synovial fibroblasts.[47] Also, anti-TNF-α antibody treatment of RA patients decreased IL-32 expression in the synovium.[48] Exacerbation of inflammation by IL-32 was confirmed in murine studies: injecting human IL-32γ into knee joints of mice led to joint swelling, inflammatory cell infiltration and cartilage damage. However, injecting human IL-32γ in the knee joints of TNF-α-deficient mice yielded no joint swelling, and the cell influx was reduced, suggesting that IL-32 activity depends, at least partly, on TNF-α.[44] The effect of human IL-32 on mouse knee joints is surprising as the mouse orthologue of IL-32 has not been identified. The mouse orthologue may not have been found because of low sequence similarity. Alternatively, the human IL-32 may act on the mouse cells through integrin via its RGD-motif.

IL-32 also has a similar relationship with IL-17, another pro-inflammatory cytokine with a role on in RA pathogenesis.[49] Both cytokines increased the expression of each other in the synovium and magnify inflammatory reactions in RA.[50] IL-32 can be induced in the FLS of RA patients by IL-17 secreted by $CD4^+$ T cells, and conversely, IL-32 expressed by FLS may induce IL-17 production by $CD4^+$ T cells.[50] IL-32 and IL-17 may signal through common intermediates, such as transcriptional coactivator p300 and death-associated protein kinase-1 (DAPK-1).[51]

In addition, IL-32 may exacerbate inflammation in RA by inducing dendritic cell maturation and IL-6 and IL-12 production that subsequently promotes Th17 and Th1 cell differentiation, respectively.[52] Although IL-32 is primarily a pro-inflammatory cytokine secreted by effector T cells besides other cell types, it was highly expressed in ex vivo CD4 + memory Treg cells (DICE).

It remains to be determined if IL-32 modulates the naïve T-cell differentiation to a pro-inflammatory Th1/Th17 or an anti-inflammatory Treg direction. Further, the cytokine may be regulated at the isoform level in effector and regulatory T cells with pro-inflammatory isoforms in the T effector cells and the anti-inflammatory isoforms in the Treg cells.

Besides promoting inflammation, cytokines contribute to the tissue damage through osteoclast differentiation in RA.[41] Osteoclasts are specialized bone resident macrophages that maintain bone homeostasis by secreting acid and lytic enzymes.[53] An excess of osteoclasts leads to bone resorption and loss. IL-32α promoted osteoclast differentiation.[54] Kim and colleagues demonstrated a significant increase in osteoclast count and activity by treating CD14 + monocytes with IL-32γ and RANKL stimulators.[55] Identifying the targets of IL-32γ in monocytes, both receptor(s) and key downstream molecules, would be useful to further understand mechanisms of IL-32 mediated bone resorption and loss.

7.2 | IL-32 in type 1 diabetes

T1D is an autoimmune disease, resulting from destruction of insulin-producing beta cells in the pancreas. Auto-reactive T cells are thought to be the key mediators of autoimmunity in T1D progression,[56] and cytokines secreted by Th1 and Th17 cells (e.g. IL-2, IL-21, IFN-γ and IL-17) have been implicated in the development of the disease.[57,58] Besides innate cytokines (e.g. GM-CSF, IL-1β, IL-7 and IL-8), levels of several T-cell-related cytokines (e.g. IL-2, IL-17, IL-21, IL-23 and IL-27) were higher in the plasma of T1D patients than controls.[59] Interestingly, the Th1 cytokines IFN-γ and IL-12 were not upregulated in patients, whereas regulatory cytokine IL-10 was upregulated in the same cohort.[59] Another group studying the plasma of T1D patients aged 15 years and over reported higher IL-1β and TNFα levels in both age groups, whereas IL-17 and IFN-γ were higher only in patients over 15 years.[60]

In a prospective longitudinal case-control study, we were first to discover that IL-32 levels are higher in PBMC of children who later developed beta-cell autoimmunity than their matched controls,[12] suggesting that IL-32 is linked to T1D disease progression. The longitudinal design of the study also revealed that IL-32 is induced before the appearance of autoantibodies (seroconversion), further suggesting that IL-32 is an early biomarker for T1D progression. Single-cell RNA-sequencing of PBMC samples revealed that that activated T and NK cells are the primary source of IL-32, which agrees with the previous findings.[2] The role of IL-32 in T1D was previously suggested in a mouse model where IL-32γ overexpression aggravated streptozotocin-induced model of T1D.[61]

Further in vitro studies in CD4 + T cells showed that IL-32γ, as well as α and β isoforms, were highly upregulated upon activation with CD3/CD28 antibodies alone. However, T cells differentiated towards Th1 cells showed higher per-cell expression of IL-32 than T cells that had been activated but not differentiated to Th1 cells. Interestingly, in activated in vitro CD4 + T cells, we observed accumulation of IL-32 inside the cells, which is consistent with the previous studies and its intracellular role. In addition, we also observed secreted IL-32 in the supernatant of Th1 cells after re-stimulation with PMA and Ionomycin.[12] However, the exact mechanism of IL-32 secretion is poorly understood and requires further studies.

Besides T cells, IL-32 in T1D may be secreted by beta cells and pancreatic epithelial cells.[62] IL-1β and IFN-γ induced IL-32 expression in the EndoC-βH1 human beta-cell

line; however, addition of IL-32γ to the cytokine cocktail did not result in additional increase in the production of pro-inflammatory cytokines, or affect the expression of endocrine marker genes (e.g. *INS*, *PDX1* and *MAFA*) or the markers of stress response genes (e.g. *ATF3*, *ATF4*, *ATF6*, *CHOP*, *HSPA5* and *sXBP1*).[12] Further, infection of beta cells with coxsackie B virus, which has been linked to the development of T1D,[63] also induced IL-32 expression.[12]

8 | CONCLUSION AND OUTLOOK

Because of its pro-inflammatory properties, IL-32 seems to be involved in several autoimmune diseases. However, to make this assertion more definitively, a better understanding of its mechanism of action is required. Identification of IL-32 receptors would be an important step. Furthermore, since the majority of isoforms are not known to be secreted, further studies are needed to understand the intracellular targets of IL-32. Besides, IL-32 may also serve as a biomarker for progression of a disease such as T1D, where the cytokine was upregulated in the peripheral blood in children progressing to the disease before appearance of T1D-associated autoantibodies. Since T cells express high levels of IL-32 and also respond to the IL-32 treatment, it would be interesting to examine the role of IL-32 in regulating differentiation of naïve CD4 + T cells towards a pro-inflammatory Th1/Th17 lineage or anti-inflammatory Treg lineage. Although IL-32 is a pro-inflammatory cytokine, certain isoforms also have anti-inflammatory properties. It would be fascinating to study the differential regulation and function of different isoforms in the context of autoimmunity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to all the aspects of the review.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Ubaid Ullah https://orcid.org/0000-0002-2539-2296

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