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Review of interleukin-32 and liver disease

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Abstract---Interleukin-32 (IL-32) is a pro-inflammatory cytokine that was first characterized in 2005. It was named IL-32 due to its potent pro-inflammatory effects. IL-32 does not contain a typical hydrophobic signal peptide at the N terminus for secretion, but IL-32 was detected in the supernatant of stimulated cells, how IL-32 is secreted is still not fully understood. Also, it is still unclear through which cell surface receptor IL-32 mediates its function. IL-32 may also have an important function as an intracellular protein⁵⁵. IL-32, via interactions with some other molecules, has been reported to be involved in various intracellular signaling. The focus of the present review is on the properties of this cytokine in liver diseases. Understanding the involvement of IL-32 in the pathogenesis of liver diseases may assist in identifying novel therapeutic strategies to mitigate or prevent liver diseases.

Keywords---IL-32, liver disease, cytokine, NAFLD.

Introduction

Interleukin-32 is a pro-inflammatory cytokine that was first characterized in 2005¹. Before that it was known as NK², a transcript induced in NK cells and T cells when activated by IL-2 and mitogens, respectively³. The IL-32 gene has no sequence homology with the other cytokine families, but was named IL-32 due to its potent pro-inflammatory effects¹. For example, recombinant human IL-32 protein induced large amounts of TNF- α in RAW264⁴. Macrophages and also activated typical cytokine-induced pathways such as NF- κ B and p38MAPK.1 IL-32 exists in numerous splice variants, generated by alternative splicing of eight exons¹. IL-32 does not contain a typical hydrophobic signal peptide at the N terminus for secretion, but IL-32 was detected in the supernatant of stimulated cells, indicating that the cytokine may be secreted¹. However, the secretory route of IL-32 was not described in detail, and now, 15 yr later, how IL-32 is secreted is still not fully understood.

Also, it is still unclear through which cell surface receptor IL-32 mediates its function. IL-32 may also have an important function as an intracellular protein⁵. IL-32, via interactions with some other molecules, has been reported to be

involved in various intracellular signaling. IL-32 α being intracellularly mediated interacts with not only paxillin, protein kinase c (PKC), and integrin, but also focal adhesion kinase 1 (FAK 1). Interaction with PKC leads to modulation of IL-6 in myelomonocytes actively expressing IL-32 α ^{6,7}. Recent studies have revealed the interactions among different isoforms of IL-32^{8,9}, as well as with other transcriptional regulators and various proteins^{6,10}. One thing that is unique to this cytokine is that a significant amount of recombinant IL-32 protein is required to activate specific cells compared to that of other cytokines. That is the reason it does not seem to be a normal cytokine and does not belong to any of the known cytokine families¹¹. The focus of the present review is on the properties of this cytokine in liver diseases. Understanding the involvement of IL-32 in the pathogenesis of liver diseases may assist in identifying novel therapeutic strategies to mitigate or prevent liver diseases.

Secretion of Interleukin-32

Most eukaryotic proteins are secreted through the conventional endoplasmic reticulum (ER)-Golgi secretory pathway, some proteins lacking a signal peptide are secreted using unconventional protein secretion (UPS), often induced in response to stress¹². IL-32 has several similarities with other stress-associated proteins such as IL-1 β , IL-33, and high mobility group that are secreted by a UPS pathway. These proteins can also be rapidly released following cell death and tissue damage and are often considered as alarmins activating the innate immune system in response to stress^{12,13,14}. One way of UPS is through membrane-bound organelles/vesicles. We established that IL-32 is secreted from malignant plasma cells in extracellular vesicles of endocytic origin. The IL-32-carrying vesicles expressed the tetraspanins CD63 and CD15, commonly used as markers for exosomes and larger vesicles of endocytic origin.²⁴ We could, however, also observe IL-32 in the Golgi compartment, thus it cannot be excluded that IL-32 is secreted from plasma cells through the ER-Golgi pathway¹⁶. IL-32 is also secreted from epithelial intestinal cells in exosomes or other types of extracellular vesicles in response to inflammatory stimuli¹⁷.

Receptor for Interleukin-32

Specific receptor for IL-32 has not been identified. Receptors for several cytokines, e.g., IL-2, IL-6, IFN γ have been identified using affinity chromatography of crude urine extract as shedding of cytokine receptors is a general phenomenon¹⁸. However, a similar analysis did not identify a receptor for IL-32. Instead, proteinase-3 (PR3) was identified as a specific and high affinity interactor of IL-32 α . PR3 is a serine protease present in membrane-bound and extracellular form in neutrophils and monocytes that cleaves several cytokines for increased activity¹⁹. Limited proteolysis of IL-32 α 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, respectively¹⁹. Furthermore, as discussed earlier, all isoforms of IL-32 contain an RGD motif through which the IL-32 α isoform binds to integrins α V β 3 and α V β 6 extracellularly²⁰.

Functions of Interleukin-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-1 β , IL-8, and IL-6 by activating nuclear factor-kappa B (NF-kB) and mitogenactivated protein kinase (MAPK) p38²¹. In monocytes, IL-32 γ enhanced the nucleotide oligomerization domain (NOD)-1- and NOD-2-induced IL-1 β and IL-6 production in a caspase 1-dependent manner but did not alter TLR-induced cytokine secretion²². Moreover, IL-32 induced the differentiation of monocytes to macrophages²³. IL-32 was required in the NOD-liganddependent dendritic cells differentiation from monocytes in leprosy²⁴. Although IL-32 has mostly been thought of as a pro-inflammatory cytokine, it is antiinflammatory in some cases. For instance, IL-32 β isoform promoted IL-10 expression in myeloid cell lines and primary monocyte-derived dendritic cells, leading to inhibition of immune responses²⁵.

Interleukin-32 and Liver disease

The main driver of progressive liver disease globally is fatty liver disease, with a 15–30% prevalence rate. It is considered to be the hepatic component of metabolic syndrome^{26,27}. The pathogenesis of NAFLD is not yet fully understood. Its existing pathophysiology proposes the multifactorial interaction of various metabolic, genetic and environmental influences, such as the gut microbiota and innate immunity interaction, mitochondrial dysfunction, abnormalities of iron metabolism, and increased fructose consumption^{28,29,30,31}, with the proliferation, dysfunction, and inflammation of adipose tissue^{32,33}.

The infiltration of adipose tissue with immune cells results in altered adipokines and promotes metabolic diseases. Moreover, chronic inflammation and insulin resistance stimulate the release of free fatty acids (FFAs) from the adipose tissue, causing hepatocellular fat deposition. Subsequently, inflammatory adipokines promote the deposition of fibrous tissue, which is the hallmark of the disease progressing to cirrhosis^{32,34}. However, the inflammatory response is also essential for tissue repair during the early phases of hepatic damage³⁵. This dual aspect of the inflammatory system may be an interesting target in the management of NAFLD. For example, interleukins such as IL-6, IL-8, IL-12, IL-18, and IL-34 have been shown to play a part in NAFLD disease^{36,37,38,39,40}.

Manal et al, observed significantly higher serum IL-32 concentrations in the NAFLD cases than in the controls. Moreover, serum IL-32 had a reliable diagnostic accuracy in differentiating NAFLD cases. Similarly, an earlier study showed that serum IL32 concentrations were higher in NAFLD cases compared to controls ($P < 0.01$)⁴¹. Furthermore, higher serum IL-32 levels were observed in patients with severe NAFLD than those without severe disease ($P < 0.01$)⁴⁴. In the same study, the inclusion of IL-32 in the ALT-AST model caused a 24% increase in AUC for the differentiation of NAFLD (AUC = 0.92 vs. 0.81)^{42,43}. Dali-Youcef et al reported that IL-32 protein levels were increased in hepatic samples of NAFLD cases. Additionally, compared to controls, IL-32 expression was raised 2.5-fold in the NAFLD cases ($P < 0.001$), while a statistically non-significant increase in IL-32 expression was detected in obese patients with normal livers (1.6-fold). Dali-

Youcef et al. also noticed a positive association between IL-32 and waist circumference, body mass index, aminotransferases, and NAFLD score, suggesting that this gene contributes to liver steatosis and metabolic syndrome⁴².

In study of Qihuan et al, it was found that hepatic IL-32 expression was increased in CHB patients and correlated with the severity of liver inflammation/fibrosis; Moreover, hepatic IL-32 expression was significantly positively correlated with serum ALT level and negatively related with serum ALB level. It was previously demonstrated that HBx could induce IL-32 expression by hepatocyte⁴⁴. It was ever reported that IL-32 induces the expressions of IL-1b, IL-6, IL-8, TNF-a, IFN-g by activating the NF-kb and p38 mitogen-activated protein (MAP) kinase pathways^{45,46}. So overexpression of IL-32 in liver of CHB suggested that it can be involved in liver inflammation/fibrosis of CHB patients. Both HBV-specific T cell response⁴⁷ and non-HBV-specific immunity 16 can result in liver damage after HBV infection.

However, IL-32 an important proinflammatory cytokine, could induce the expressions of IL-1b, TNF-a, IL-6 by monocyte, macrophage^{45,45}. In the same study, it was found that hepatic IL-32 expression was increased in CHB patients. As a result, a cascade of intrahepatic cytokine network events during HBV infection is initiated, and liver is damaged. Their results for the major proinflammatory function of IL-32 in chronic HBV infection were in accordance with research of Shioya et al. Shioya et al ⁴⁸. demonstrated that inflammatory responses in the affected mucosa of patients with inflammatory bowel disease may be enlarged by a consecutive loop of IL-32-induced TNF-a secretion from monocytes and TNF-a-stimulated IL-32 secretion from epithelial cells. These results do emphasize the correlation of IL-32 with inflammation and the other proinflammatory cytokines and chemokines.

Conclusions

Despite the relatively low number of studies published to date, However, previous studies have shown that there is a link between abnormal levels of IL-32 and liver disease.

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