Comparison of the Seven Interleukin-32 Isoforms’ Biological Activities: IL-32\(^\text{q}\) Possesses the Most Dominant Biological Activity

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Cytokines are significantly associated with the homeostasis of immune responses in health and disease. Interleukin-32 (IL-32) is a cytokine originally discovered in natural killer cell transcript 4. IL-32 with different disorders has been described in terms of pathogenesis and the progression of diseases. Clinical studies have investigated IL-32 under various conditions, such as viral infection, autoimmune diseases, inflammatory diseases, certain types of cancer, vascular disease, and pulmonary diseases. The high expression of IL-32 was identified in different tissues with various diseases and found to have multiple transcripts of up to seven isoforms. However, the purification and biological activities of these isoforms have not been investigated yet. Therefore, in this study, we purified and compared the biological activity of recombinant IL-32 (rIL-32) isoforms. This is the first time for seven rIL-32 isoforms (\(\alpha, \beta, \delta, \gamma, \epsilon, \zeta,\) and \(\theta\)) to be cloned and purified using an \textit{Escherichia coli} expression system. Next, we evaluate the biological activities of these seven rIL-32 isoforms, which were used to treat different types of cells by assessing the levels of inflammatory cytokine production. The results revealed that rIL-32\(^\text{q}\) possessed the most dominant biological activity in both immune and non-immune cells.

Keywords: interleukin-32, recombinant protein, isoforms, IL-32\(^\text{q}\), inflammatory cytokine
INTRODUCTION

Interleukin-32 (IL-32) cytokine was cloned in 1992 from natural killer cell and was formerly named natural killer cell transcript 4 (NK4). NK4 was renamed IL-32 in 2005 because it has a cytokine property (1, 2). It was found to induce several inflammatory cytokines, such as tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP2), interleukin-8 (IL-8), and interleukin-1 beta (IL-1β), and IL-32 acts like a proinflammatory cytokine (1–3).

Nevertheless, since its discovery, much knowledge remains to be determined. For the most part, its specific surface receptor has yet to be defined. Proteinase 3 (PR3) binds to IL-32 with very high affinity (4). PR3 is a serine protease produced from neutrophils as an enzyme, whereas it is also expressed on the membrane of monocytes. The possibility of IL-32 binding to integrins has been suggested (5), and this result was based on its amino acid composition containing an RGD motif, which ubiquitously presents in various genes. The IL-32 amino acid sequence has no known cytokine homolog; in addition, IL-32 was detected in most mammals except rodents (6).

A previous study reported that the IL-32 gene is composed of eight exons and presents within human chromosome 16p13.3 (1). According to its alternating splicing sites, more than seven transcripts have been suggested. However, seven isoforms with nine exons were described to be translated from its messenger RNA transcript (7). These isoforms are IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, and IL-32θ. As each isoform was discovered separately, the cell type, condition, and isoform function were varied. IL-32α, IL-32β, IL-32γ, and IL-32δ were mainly identified in IL-2-stimulated human NK cells; on the other hand, IL-32ε and IL-32ζ were found to be expressed in activated T cells (8). Lastly, IL-32θ was discovered from dendritic cells and Jurkat cells of human leukemia T cell line (9). These isoforms exhibited distinct effects in different conditions. Among the seven IL-32 isoforms, IL-32γ is the most-studied isoform, which also has the longest amino acid sequence.

IL-32 plays a vital modulator role in the pathogenesis of different diseases. Its involvement has been reported in various cancers, infections, and autoimmune and inflammatory disorders (6, 10, 11). Most autoimmune and inflammatory diseases associated with IL-32 are rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, chronic obstructive pulmonary disease (COPD), and asthma (3, 7, 10, 12–14). However, these clinical studies determined the levels of circulating IL-32 and then compared the patients to healthy controls. These studies fail to characterize the differences in IL-32 isoforms. However, the protein identification of each IL-32 isoform is subjected to a significant limitation because of the lack of a specific antibody to detect the IL-32 variant. Moreover, IL-32 secreted proteins are not easily purified since the structures of IL-32 isoforms are not thoroughly appraised.

At present, we were able to purify seven rIL-32 isoforms: with IL-32α, IL-32β, IL-32δ, and IL-32γ purified in our previous study, whereas IL-32ε, IL-32ζ, and IL-32θ were purified for the first time in this study. Next, we assessed the biological activities of the seven rIL-32 isoforms in various cells by examining the production of inflammatory cytokines, such as IL-6, IL-8, TNFα, and MIP2. Seven rIL-32 isoforms show a different biological activity regarding different cell types.

MATERIALS AND METHODS

Isoform Cloning and Expression

All seven isoforms were cloned into pPROEX/HTa from Takara (Shiga, Japan) as previously described (8). IL-32α, IL-32β, IL-32δ, IL-32ε, IL-32ζ, and IL-32θ isoforms were cloned earlier, and the remaining three IL-32c, IL-32γ, and IL-32ω isoforms were constructed in this study for the first time using the closest isoform as the template, as shown in Figure 1B.

Briefly, pPROEX/HTa IL-32β plasmid was used to construct IL-32γ and IL-32θ. Then, IL-32θ was used to construct IL-32c (Figure 1C). For the construction of a new isoform plasmid vector, we used overlap extension PCR with primers as indicated in Figure 1C. All PCR products were designed to have EcoRI and XbaI restriction enzyme sites in their 5′ and 3′ ends. Next, the PCR products were ligated into an expression vector using EcoRI and XbaI restriction enzymes (Takara) and confirmed by DNA sequencing analysis in Cosmogen (Seoul, Korea). These expression vectors were transformed into BL21-Codon Plus (San Diego, CA, USA) by heat shock method.

Recombinant Protein Expression and Purification

Seven recombinant rIL-32 (α, β, γ, δ, ε, ζ, and θ) proteins were expressed in E. coli with 4-h isopropyl β-D-1-thiogalactopyranoside induction at 37°C. rIL-32β, rIL-32γ, and rIL-32θ were purified with Ni-NTA agarose from Qiagen (Hilden, Germany), and the others were purified with TALON® Magnetic Bead (Takara) using his®-tag at the N-terminus of rIL-32 isoform proteins. Among the affinity-purified proteins, rIL-32β, rIL-32γ, and rIL-32θ were subjected to a high-performance liquid chromatography column from Grace (Stockbridge, GA), and rIL-32α, rIL-32ε, rIL-32ζ, and rIL-32θ were subjected to an anion exchange column (HiTrap Q FF, 1 ml) from GE Healthcare (Chicago, IL, USA). After that, we checked their concentration by silver staining, Bradford assay, and BCA assay. Next, to check the bands of purified rIL-32 isoform proteins, we did western blotting with mouse anti-his®-tag mAb from R&D system (Minneapolis, MN, USA). The rIL-32 proteins were tested with a LAL chromogenic endotoxin quantitation kit from Thermo Fisher (Waltham, MA, USA). The endotoxin level was below 0.5 EU per 1 μg of rIL-32 protein, which is approximately 0.05 ng in 1 μg of rIL-32.

Gene Expression Analysis

The expression levels of IL-32 in normal tissues were identified using GTEx Portal (https://www.gtexportal.org/home/).

Cell Culture and Cytokine Assays

THP-1 and Raw 264.7, A459 cell lines were obtained from ATCC (Manassas, VA, USA). The THP-1 monocytes and Raw 264.7 cells were cultured in RPMI 1640 medium supplemented with...
10% fetal bovine serum (FBS), 100 μg/ml penicillin, and 100 μg/ml streptomycin. A549 was cultured in Ham’s F12K medium containing the same reagents. Mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) medium containing the same reagents. All cell culture media were from Welgene Biotech (Taipei, Taiwan). The culture condition was as follows: under humidified 5% CO₂ at 37°C.

THP-1 (2.5 × 10⁴/well), Raw 264.7 (5.0 × 10⁴/well), and A549 (2.5 × 10⁴/well) were seeded in a 96-well plate of 100 μl volume. THP-1 and Raw 264.7 were treated with different concentrations of purified rIL-32 isoforms (11.1, 33.3, and 100 ng/ml) in 100-μl-volume media, and the control was treated with the media alone. A549 and MEFs were treated with different concentrations of purified rIL-32 isoforms (100, 200, and 1,000 ng/ml) in 100-μl-volume media, and the control was treated with media alone. After 18 h of stimulation, the supernatants of THP-1 and Raw 264.7 were assessed for human IL-8 and mouse TNFα measurements, respectively. The A549 and MEF supernatants were assessed for human and mouse IL-6 measurements, respectively. All tested cytokines were determined by ELISA kits (R&D system).

**Cell Isolation From Mouse and Cytokine Assays**

To verify that rIL-32 isoforms induced various cytokines in primary cells, we prepared splenocytes, bone marrow cells, and lung cells from C57BL/6 from Orient Bio (Seoul, Korea). All animal procedures were reviewed and approved by the Konkuk University Institutional Animal Care Committee. A C57BL/6 mouse was dissected, and the spleen, bone, and lung were isolated. We mashed the spleens and collected bone marrow cells from bones. These were centrifuged, washed with Dulbecco’s phosphate-buffered saline, and suspended in RPMI1640 medium supplemented with 10% FBS, 100 μg/ml

![Schematic representations of the seven IL-32 isoforms.](image)
penicillin, and 100 μg/ml streptomycin. In the case of lung cells, these were chopped, centrifuged, treated with collagenase V, and suspended in RPMI 1640 medium supplemented with 10% FBS, 100 μg/ml penicillin, and 100 μg/ml streptomycin. The MEF cells were prepared as follows: the fetus was isolated at 13.5 days of pregnancy. The fetus was chopped and digested with trypsin and DNase 1 and then suspended and cultured in DMEM.

RESULTS

The Construction, Expression, and Purification of Seven rIL-32 Isoforms

Seven IL-32 isoforms were constructed and cloned as shown in Figure 1A. IL-32 was divided into 9 small domains, and domain 8 is the longest. The amino acid sequence of each domain was illustrated with different colors, as shown in Figure 1B, corresponding to the color of the domain in Figure 1A. Each isoform of complete open reading frame in pPROEX/HTa E. coli had its expression vector confirmed by DNA sequencing. Multi-step (his6-tag purification and ion-exchange chromatography or high-performance liquid chromatography) purification was employed to obtain seven pure isoforms of rIL-32 protein. Figure 2A shows the 10% SDS-PAGE analysis of rIL-32 isoforms, with the dominant bands of each isoform corresponding to its theoretical molecular size as follows: IL-32α: 19.8 kDa, IL-32β1: 25.5 kDa, IL-32γ: 31.5 kDa, IL-32β2: 24.2 kDa, IL-32c: 20.7 kDa, IL-32ζ: 24.4 kDa, and IL-32θ: 23.0 kDa, plus 5.4 kDa of N-terminus his6-tag. All seven rIL-32 isoforms were migrated slowly; therefore, the molecular weight in silver staining was slightly higher than the actual molecular weight of each rIL-32 isoform. In addition to this, some rIL-32 isoforms appeared as a dimer in multiple bands. To confirm whether these bands were purified rIL-32 from E. coli or not, we did a western blot analysis using mouse anti-his6 tag mAb. As shown in Figure 2B, all protein bands in silver staining were found to be bound with mouse anti-his6 tag mAb to confirm the purity of the final seven rIL-32 isoform proteins.

Gene Expression of IL-32 in Different Cell Types (Using GTEx)

It has been reported that the expression of IL-32 cytokine is increased in a variety of inflammatory autoimmune diseases and certain infections and cancers. We evaluated the expression of IL-32 in normal tissues using GTEx portal. Additionally, IL-32 is expressed in many cell types, including immune and non-immune cells, exhibiting different activities, which may be due to differences in cell types and/or stimulus and different isoform expressions related to cell types. However, a comparison of the activity of IL-32 isoforms has not been elucidated. IL-32 expression in normal tissues revealed that the highest expression of IL-32 was found in the spleen, followed by Epstein–Barr virus-transformed lymphocytes and then lung tissues (Figure 3). Therefore, we compared the activity of the seven IL-32 isoforms in several cell types, including immune cells, lung cells, and fibroblasts. THP-1 (human-derived monocytes) and Raw264.7 (mouse-derived monocytes/macrophages) cell lines were used to evaluate the biological activity of rIL-32 isoforms. Primary mouse bone marrow and splenocytes were also isolated to evaluate the biological activity of rIL-32 isoforms. rIL-32 promotes the differentiation of monocytes into macrophage-like cells, inducing proinflammatory cytokines such as TNFα, IL-6, and IL-8 (15). Therefore, we treated the selected cell types with the seven rIL-32 isoforms and...
measured the cytokine productions to evaluate the biological activity of each rIL-32 isoform and determine the dominant isoform in each cell type.

**Recombinant IL-32β, -γ, and -θ Induced Cytokines in Immune Cells**

The biological activity of the seven purified rIL-32 isoforms was assessed in immune cells. First, THP-1 and Raw 264.7 were stimulated with the seven rIL-32 isoforms, and a cell culture supernatant was used to assess the levels of IL-8 and TNFα production, respectively (Figure 4). Moreover, mouse isolated primary bone marrow and splenocytes were stimulated with the seven rIL-32 isoforms. Next, IL-6 from bone marrow as well as IL-6, TNFα, and MIP2 from splenocytes were assessed (Figure 5). The production of the measured cytokines was significantly increased by three rIL-32 isoforms, which were rIL-32θ, -γ, and -β isoforms, in a dose-dependent manner. These results were consistent in immune cell lines (THP-1 and Raw 264.7) and primary mouse immune cells (splenocytes and bone marrow cells). At the same time, the remaining four (rIL-32α, -δ, -ε, and -ζ) isoforms have a weak or no activity in cytokine production.

In the case of THP-1 cells that were stimulated with different concentrations of rIL-32θ, these showed ±4 folds of IL-8 production compared to non-stimulated cells, and all three concentrations (11.1, 33.3, and 100 ng/ml) were significantly increasing the IL-8 levels, followed by rIL-32γ with concentrations of 33.3 and 100 ng/ml and then rIL-32β with a higher concentration only 100 ng/ml, which thus significantly induced IL-8 production. Interestingly, only rIL-32θ induced a significant amount of IL-8 production at a low concentration. Thus, rIL-32θ was considered the most potent rIL-32 isoform in this cell line. On the other hand, both rIL-32α and rIL-32δ showed ±1 ng/ml production of IL-8 at their highest concentration of 100 ng/ml. rIL-32ɛ and rIL-32ζ did not induce IL-8 production (Figure 4A).

**FIGURE 4** | Biological activities of seven rIL-32 isoforms in immune cell lines. rIL-32 isoforms with different concentrations were treated in cells for 18 h. The levels of IL-8 and TNFα were measured in the supernatant of THP-1 (A) and Raw 264.7 (B), respectively, by using ELISA. The bar graph represents the level of cytokines, mean ± SEM. Statistical testing was performed using two-way ANOVA followed by Tukey’s post-hoc analysis. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 as compared to no treatment control within the same isoform treatment; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 as compared to other displayed isoform symbols treated with the same concentration.
The results from Raw 264.7 cells were similar to the THP-1 results, with one difference in the dominant isoform, which was rIL-32\(g\) showing ±2.5 folds of TNF\(\alpha\) production instead compared to non-stimulated cells at the concentrations of 33.3 and 100 ng/ml. However, rIL-32\(q\) and rIL-32\(g\) induced significant TNF\(\alpha\) production at 100 ng/ml (Figure 4B).

Next, the seven rIL-32 isoforms were treated in primary mouse bone marrow cells and splenocytes. The effect of each rIL-32 isoform on the production of cytokines is shown in Figure 5. In bone marrow cells, all rIL-32 isoforms at a low concentration (11.1 ng/ml) did not induce IL-6 production. Only two isoforms (rIL-32\(g\) and rIL-32\(a\)) induced the production of IL-6 at 33.3 and 100 ng/ml. However, the levels of IL-6 were significantly higher at 100 ng/ml of rIL-32\(q\), followed by rIL-32\(g\) isoform (Figure 5A). Concurrently, rIL-32\(g\) and rIL-32\(a\) significantly induced higher IL-6, TNF\(\alpha\), and MIP2 in the primary mouse splenocytes as shown in Figures 5B–D, respectively.

Recombinant IL-32\(\alpha\), \(\beta\), \(\delta\), and \(\theta\) Induced Cytokines in Lung Cells

Biological activity was assessed in human A549 lung cells and primary mouse lung cells. Both cells were treated with rIL-32 seven isoforms of different concentrations for 18 h; then, the levels of IL-6 were assessed (Figure 6). In both lung cells, rIL-32\(\theta\) showed a highly significant production of IL-6 than the other six isoforms in a concentration-dependent manner. However, unlike immune cells, rIL-32\(\gamma\) showed weak or no biological activities in A549 (Figure 6A) and mouse isolated lung cells (Figure 6B), respectively. Moreover, rIL-32\(\delta\), \(\beta\), and \(-\alpha\) demonstrated a significant biological activity at high concentrations, in terms of IL-6 production, only on A549 cells. The remaining isoforms, rIL-32\(c\) and rIL-32\(z\), still have a very weak activity on A549 cells.

Recombinant IL-32\(\beta\), \(\delta\), and \(-\theta\) Induced Cytokines in Fibroblast Cells

Fibroblasts are cells that are mainly accountable for maintaining the extracellular matrix and are found within many tissues and organs such as the skin and lungs. Therefore, we measured the production of IL-6 in MEF cells treated with different concentrations of the seven isoforms to assess their biological activity (Figure 7). Like the immune and lung cells, rIL-32\(\theta\) showed the highest production of IL-6. Nevertheless, a significant induction was found only with the high concentration of isoform at 1,000 ng/ml. Moreover, IL-32\(\delta\) and IL-32\(\beta\) also showed a significant production of IL-6 following rIL-32\(\theta\) at the high concentration of 1,000 ng/ml. rIL-32\(\alpha\) and rIL-32\(\gamma\) showed...
a slightly non-significant production of IL-6, whereas rIL-32c and rIL-32ζ isoforms did not induce cytokine production.

DISCUSSION

IL-32 is a novel multifunctional cytokine involved in various cell functions, differentiation, pro- or anti-inflammatory cytokines stimulation, and apoptosis (15–22). This cytokine promotes the induction of crucial inflammatory cytokines such as IL-1β, TNFα, IL-6, IL-8, and MIP2 (1, 2, 15, 23). Its expression engages numerous pathogenesis disorders, including inflammatory, autoimmune diseases, cancers, and infections (6, 24–26). IL-32 is found to come up with different splice variants (7, 27). However, there are limitations on IL-32 isoform characterization and correlation to define biological processes or disease conditions.

In this study, we were able to purify seven rIL-32 isoforms and evaluate their biological activity in different cell types, which may shed light on the specific activities of these seven IL-32 isoforms. Among them, four rIL-32 isoforms (IL-32α, -β, -γ, and -δ) were previously purified (8). Moreover, the remaining three rIL-32 isoforms (IL-32c, -ζ, and -θ) were successfully constructed and purified for the first time in this study.

The expression of IL-32 in normal tissue revealed a high expression among various cell types, e.g., lung cells, fibroblasts, and immune cells, including monocytes and bone marrow (Figure 3). IL-32 is highly associated as well with disease conditions relating to these cell types, like rheumatoid arthritis, COPD, asthma, atopic dermatitis (AD), and certain cancers (6, 10, 12, 24, 28, 29). Therefore, we investigated the differences in the biological activity of the seven IL-32 isoforms within immune cells, lung cells, and fibroblasts. This study illustrated the need for a fundamental activity study regarding each IL-32 isoform.

So far, the expression of IL-32 has been correlated with numerous autoimmune diseases, among them RA and IBD that were the most-studied conditions in this regard. In the case of RA and compared to both healthy controls and patients with osteoarthritis, IL-32 expression was higher in RA patients (30); moreover, the synovial biopsies of RA patients exhibit a reduction of IL-32 upon anti-TNFα treatment. This interchange between IL-32 and TNFα suggests an intensification of inflammatory processes in RA (18). Regarding IBD, IL-32 has been suggested to have a role in the pathogenesis of IBD as it promotes the production of TNFα, IL-6, and IL-1β cytokines (31). To a lesser extent, patients with autoimmune diseases, including psoriasis, granulomatosis with polyangiitis, myasthenia graves, and type 2 diabetes, have also demonstrated a higher serum level of IL-32 than healthy controls (32–34). This difference was linked to disorder severity, suggesting its usefulness in being an inflammatory marker and outcome predictor.

More recently, IL-32 is also involved in type 1 diabetes; its mRNA levels in beta-cells were higher than in those in control subjects (35). These results are in line with the outcome of Jhun...
et al., who found that IL-32, specifically the gamma isoform, hastens streptozotocin-induced type 1 diabetes (36). In addition to autoimmune diseases, IL-32 is involved in respiratory inflammation conditions, such as COPD and asthma (12–14, 29, 37, 38). Its expression in lung tissue is enhanced in COPD patients and was associated with the obstruction degree of airflow in vivo (12). Besides this, IL-32 was found to play a role in gastric inflammation and cancer (39, 40), altogether signifying the execution of IL-32 in several inflammatory conditions with different patterns that could be explained by the existence of different isoforms that play different roles. Nevertheless, many of these studies fail to convey the IL-32 isoforms concerning disease conditions.

Lately, with increasing inconsistent reports regarding the role of IL-32, there is a large agreement that these different functions may relate to the different IL-32 isoforms. As mentioned earlier, most of the previous studies assessed the level of IL-32 with lack of specific isoform consideration. However, limited studies have demonstrated a few properties of some isoforms—for example, IL-32α has shown pro- and anti-inflammatory properties as it induces pro-inflammatory cytokine expression, thus suppressing its inflammatory role in the spinal cord. Besides this, the ability of IL-32α to promote the differentiation of osteoclast has been reported (17).

IL-32β also has both pro- and anti-inflammatory properties; it induces cytokine production of both IL-10 and TNFα in phorbol-12-myristate-13-acetate-stimulated cells, K562, and THP-1, respectively (1, 41, 42). In addition, this isoform also improves the adhesion ability of inflammatory cells to activate endothelial cells along with the consequent induction of proinflammatory cytokines. Therefore, it is involved in vascular inflammation propagation and the modulation of lipid accumulation (43, 44).

The longest isoform, IL-32γ, exhibits mainly a pro-inflammatory property and accordingly induces pro-inflammatory cytokine expression. Moreover, IL-32γ promotes the migration of activated T cells via chemokine (C-C motif) ligand 5 (CCL5) production in dendritic cells (DCs), stimulates the maturation and activation of DCs, and therefore increases the production of IL-12 and IL-6 (45, 46). In ankylosing spondylitis joint, IL-32γ plays an enhancement role in the differentiation of osteoblast (47). In RA patients, the level of IL-32γ was found to be upregulated significantly in both CD14+ monocytes and synovial membrane (16, 48). Therefore, it has been suggested that this isoform activates osteoclasts and, subsequently, tissue resection. Furthermore, IL-32γ has shown a potent antiviral activity versus several viruses, specifically influenza A virus, vesicular stomatitis virus, herpes simplex virus 2, and human immunodeficiency virus (49–52).

IL-32δ is another isoform that generally demonstrates a proinflammatory property; it inhibits the production of IL-10. This inhibition occurs through the modulation of IL-32δ; thus, this observation reveals that IL-32 is controlled by its isoforms (53). On the contrary, IL-32δ has mainly anti-inflammatory effects and has an inhibitory role on monocyte differentiation (41, 54). In patients with acute myeloid leukemia, IL-32δ regulates the production of TNFα negatively (55). In addition, IL-32δ negatively regulates CCL5 expression, an inflammatory chemokine secreted in several conditions such as viral infection and cancer, at both mRNA and protein levels. This data suggests the intracellular modulator role of IL-32δ under inflammation (56). Additionally, the isoform of IL-32δ has been found to suppress epithelial-mesenchymal transition, resulting in inhibition of invasion and migration of colon cancer cells under in vitro and in vivo assessments (57). Lastly, for IL-32ε, its transcript was elevated in the IBD mucosa, thus suggesting a protective activity (58). However, the present study showed that the IL-32 isoform has the most prominent activity among the seven IL-32 isoforms.

There has been a widespread acceptance that IL-32γ is the most biologically active isoform (6, 8, 19, 26), as recent results suggest such interpretation. This conclusion is probably attributable to IL-32γ as it is the most-studied isoform. Here our data is in line with the interpretation regarding IL-32γ, specifically within the immune cells along with IL-32 isoform. In more detail, we observed a higher activity of IL-32 isoform in human-derived monocytes, THP-1, followed by the IL-32γ isoform (Figure 4A). However, in mouse-derived cells, IL-32γ exhibits maximum activity. Remarkably, IL-32β activity was directly following IL-32γ and IL-32α among the tested immune cells (Figures 4 and 5). It is noteworthy that IL-32 switches between its isoforms under certain conditions were reported to reduce the inflammation as a safety control. This shift of transcripts has been indicated between IL-32γ and IL-32β isoforms (19). A similar shifting may be the case with IL-32δ to reduce its potent activity. More investigation is needed to confirm this suggestion and thus specify the key exon/domain/peptide signal responsible for the splicing change in both cases as well as examine the possibility of dimerization of IL-32β to reduce its section.

So far, few studies have been conducted on IL-32 compared to the IL-32γ isoform. Interestingly, IL-32δ was the most active isoform in most cell types except in mouse Raw 264.7 and splenocyte (Figures 4B and 5B). These in vitro results using a human-derived cell line (A549) showed four active isoforms with a difference in activity as reflected by the different levels of IL-6 production; these are IL-32δ, -δ, -β, and -α, in descending order. In comparison, ex vivo results using mouse-derived cells demonstrated a significant IL-6 production only with the IL-32γ isoform. In addition, IL-6 production in MEF showed that IL-32δ has the highest significant activity, followed by IL-32β and IL-32δ with comparable results. As mentioned above, IL-32δ has been suggested to play an intracellular modulatory role in breast cancer cells (56, 59). On the other hand, a study conducted on asthma patients showed a lower IL-32γ compared to healthy controls (37) as well as rIL-32γ that showed a negative regulatory effect in an asthma mouse model (14). However, this report did not consider the behavior of the IL-32δ isoform.

Inflammatory skin conditions have revealed the changes in levels of IL-32 with restricted and conflicting data regarding isoforms. A study comparing patients with asthma, psoriasis, and AD to healthy subjects found that IL-32 was higher in asthmatic and AD patients’ serum (60). They suggest that the release of IL-32 is mainly from apoptotic cells in both conditions, which is also in line with their in vitro results. Therefore, they declared the usefulness of using IL-32 serum levels in diagnosis to examine patients with AD or asthma.
In addition, they mentioned the possibility of targeting IL-32 as a therapeutic purpose. There are some contradicting reports of Al-Shobaili et al., Meyer et al., and Lee et al. on the one hand, whereas Al-Shobaili et al. and Meyer et al. have found that the levels of IL-32 are increasing in psoriasis and AD, respectively (22, 61). Lee et al. reported that IL-32 exhibits a suppressor role for AD (60). Thus far, additional studies are needed to explain the role of these isoforms in different stages and different stimuli and their impact on each other.

In summary, we purified seven rIL-32 isoforms using the E. coli expression system and evaluated their biological activities using various cell types. Along with rIL-32γ, rIL-32β revealed similar or higher activities in all tested cells. However, the behavior of IL-32 isoforms could be different at baseline and other conditions, as it may be influenced by many factors, such as different stimuli, health/disease conditions, cell type, and genetic background. Moreover, rIL-32c and rIL-32z both showed little or no activity in the tested cells. Nevertheless, our results indicated the necessity to illuminate each rIL-32 isoform. Therefore, both mRNA and protein levels, in the forthcoming studies, should be considered. Furthermore, specific monoclonal antibodies that recognize each isoform are needed to accomplish this need, such as in the case of IL-32γ (62).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

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ETHICS STATEMENT

All animal procedures were reviewed and approved by the Konkuk University Institutional Animal Care Committee.

AUTHOR CONTRIBUTIONS

SS and SL designed the study, analyzed the data, and performed the experiments. SS, SL, SK, TTN, AT, and JH, performed the experiments. HJ, YL, SCY, and Y-GK analyzed the data. Funding acquisition was carried out by HJ, H-YP, S-YK, Y-GK, and SK. SS and YH examined the data. AT edited the manuscript. SK designed the study, supervised the project, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This paper was written as part of Konkuk University’s research support program for its faculty on sabbatical leave in 2022. This work was supported by the National Research Foundation of Korea (NRF-2021R1F1A1057397). This research was supported by the Main Research Program (E0210602-02) of the Korea Food Research Institute (KFRI), funded by the Ministry of Science and ICT. S-YK and Y-GK were supported by NRF-2021M3A9G1026605. SL was supported by NRF-2019R1I1A1A01057699.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.
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