

Th22 Cells Promote in vitro Proliferation and Cytokine Secretion in HaCaT and HRMC cells via Production of IL-22 and Stimulation of PI3K/AKT Signaling

Ling Zhao

Jilin University

Dejun Sun

Jilin University

Tao Liu

Jilin University

Zhenyu Jiang

Jilin University

Zhuang Ye (✉ zhuangye1987@jlu.edu.cn)

Jilin University

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Abstract

Background: T helper (Th) 22 cells function in the pathogenesis of systemic lupus erythematosus (SLE), but their exact role remains unclear. We examined in vitro interactions of Th22 cells with different target cells.

Methods: Flow cytometry was used to isolate Th22 cells from the peripheral blood mononuclear cells (PBMCs) of 5 healthy subjects. The optimal co-culture ratio of effector:target (E:T) cells and the optimal co-culture concentration of recombinant interleukin (rIL) -22 were determined. The levels of IL-22, proliferation of target cells, and the levels of proinflammatory cytokines in different culture systems with human keratinocyte cells (HaCaT) and human renal mesangial cells (HRMC) were determined. Western blotting was used to assess changes in the levels of proteins in the PI3K/AKT pathway.

Results: The levels of IL-22 were greater when HaCaT and HRMC cells were co-cultured with Th22 cells or rIL-22, and co-culturing with contact had a greater effect than co-culturing without contact. The levels of cytokines were also significantly greater when target cells were co-cultured with Th22 cells. IL-22mAb reversed the effect of IL-22. Th22 cells significantly activated PI3K/AKT signaling in HaCaT and HRMC cells; LY294002 (an inhibitor of this pathway) reversed this effect and also downregulated the levels of proinflammatory cytokines; IL-22mAb had a similar but weaker effect.

Conclusions: Our results suggest that Th22 cells promote cell proliferation and secretion of pro-inflammatory cytokines of their target cells via IL-22-mediated stimulation of PI3K/AKT signaling.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that is characterized by the presence of numerous serum autoantibodies and the involvement of multiple organs, including the kidneys, skin, central nervous system, and joints(1). Abnormal T helper (Th) cells and proinflammatory cytokines produced by these cells contribute to the pathogenesis of SLE(2–5).

Researchers first identified the Th22 subset as a CD4 + T cell subset in 2009(6). Th22 cells secrete IL-22, but do not secrete IL-17, IL-23R, CCL20, CD161 (Th17 markers), IFN- γ (Th1 marker), or IL-4 (Th2 marker) (7). The development of Th22 cells depends on the transcription factor aryl hydrocarbon receptor (AHR) (6). On the other hand, because Th22 cells express the chemokine receptor CCR6 and the skin-homing receptors CCR4 and CCR10, they can be defined as CCR6⁺CCR4⁺CCR10⁺ cells(8). Th22 cells also express all the typical markers of Th cells, such as CD3 and CD4(9).

There is evidence that Th22 cells function in many inflammatory and autoimmune disorders, including SLE(10, 11), psoriasis(12), and rheumatoid arthritis (RA)(13). Similar to other Th cell subsets, the function of Th22 cells is mainly attributed to their secretion of a lineage-specific functional molecule, in this case IL-22. Th22 cells produce about 50% of the IL22 in the peripheral blood of humans(6). Other cells (T cells(14), NK and NKT cells(15, 16), and non-lymphoid cells(17–19)) and tissues (liver, lung, skin, kidney,

synovium, and eye(20–22)) that function in innate and acquired immunity also produce IL22. IL22 is in the IL10 family. The components of the heterodimeric IL22 receptor complex consist of IL22R1 and IL10R2. The IL22 receptor is in cytokine receptor family class 2 (CRF2). Most tissues express IL10R2, but only non-hematopoietic cells, such as keratinocytes, hepatocytes, and epithelial cells, express IL22R1 (23). The cellular and anatomical distribution of IL22 and its receptor suggest that the target cells of IL-22 are common in the cells and tissues mentioned above.

IL-22 has multiple functions. It can promote the repair of local tissue damage, but can also promote pathogenic inflammation. Previous studies showed that IL-22 has a protective effect by mediating epithelial tissue repair and regeneration, inhibiting apoptosis, and stimulating progenitor cells in hepatitis, influenza, and pneumonia (24–26). However, IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility of keratinocytes in patients with psoriasis(27). IL-22 also has synergistic interactions with multiple cytokines, such as TNF- α , IL-17, and IL-20, and the resulting cytokine network affects the progression of many different diseases(28).

Many researchers have examined the relationship of Th22 cells with IL-22 because they can have protective effects in some diseases and pathological effects in other diseases. Our previous study indicated that patients with SLE (relative to healthy controls) had an increased abundance of Th22 cells and an elevated plasma level of IL-22 in their peripheral blood. We also found positive correlations of Th22 cell abundance, plasma IL-22 level, and disease activity(29). Yang et al. found that SLE patients with skin involvement had elevated levels of IL-22 and Th22 cells, and that the proportion of Th22 cells was positively correlated with plasma IL-22 level(30). They also found that IL-22 levels in the serum and kidney were significantly higher in patients with lupus nephritis (LN) than healthy controls. The serum and renal levels of IL-22 in MRL/lpr mice (a model of systemic autoimmunity) increased significantly over time(11). However, some clinical observations found that the peripheral blood of patients with SLE had lower levels of IL-22 than healthy controls(31). Our recent study indicated that SLE patients with skin and renal involvement had increased levels of Th22 cells and an increased concentration of plasma IL-22(32). Furthermore, we used immunofluorescence analysis with confocal microscopy to detect the distribution of Th22 cells and IL-22 receptors in the organs involved in SLE (kidney and skin). The results indicate that IL-22 appears to target kidney mesangial cells and skin basal keratinocytes(33).

There is very limited research on the interactions of Th22 cells with target cells. Thus, we used an in vitro co-culture system to examine the potential roles and mechanism of Th22 cells on the proliferation and the secretion of pro-inflammatory cytokines by human keratinocyte (HaCaT) cells and human renal mesangial cells (HRMC).

Results

IL-22 increases proliferation and production of proinflammatory cytokines in HaCaT and HRMC cells

Our initial experiments with HaCaT cells and HRMA cells indicated that addition of increasing levels of rIL-22 increased cell proliferation (Fig. 1a, e). Cell proliferation of HaCaT cells was significantly greater in all treatment groups than the control group, and the 30 ng/mL and 100 ng/mL groups were similar. Cell proliferation of HRMC cells was not significantly different for the control, 10 ng/mL, and 30 ng/mL groups, but the 100 ng/mL group had significantly increased proliferation. Treatment with increasing levels of rIL-22 also increased the levels of 3 proinflammatory cytokines in HaCaT cells (Fig. 1b-d) and HRMA cells (Fig. 1f-h). Based on these results, we used an rIL-22 concentration of 100 ng/mL for the subsequent co-culture experiments.

Co-culture of Th22 cells with HaCaT or HRMC cells increases the levels of IL-22 and proinflammatory cytokines

Our co-culture experiments with the two target cells indicated that the level of IL-22 increased as ratio of Effector: Target (E: T) cells increased, and that the level of IL-22 was greatest for a 4:1 ratio of E:T cells (Fig. 2a, f). Analysis of cell proliferation indicated no significant effect in HaCaT cells until the E:T ratio was 4:1 (Fig. 2b), although HRMC cells had significantly greater proliferation at an E:T ratio of 0.5:1 (Fig. 2g). In addition, the concentrations of the three proinflammatory cytokines increased as the E:T ratio increased up to 4:1 for both target cells (Fig. 2c-e and h-j). Based on these results, we used a 4:1 ratio of E:T cells for subsequent co-culture experiments.

Th22 cells induce cytokine secretion by HaCat and HRMC cells in the Transwell system

Adding Th22 cells or rIL-22 to HaCaT cells significantly increased the level of IL22 and cell proliferation, and these responses were even greater when the effector and target cells were co-cultured with contact than without contact (Fig. 3a, b). The changes in the levels of pro-inflammatory cytokines in the supernatants were similarly increased (Fig. 3c-e). However, only the TNF- α level was greater when the E and T cells were co-cultured with contact than without contact. Moreover, addition of IL-22mAb reversed these effects (Fig. 3b), indicating that promotion of HaCaT cell proliferation was IL-22 dependent. Addition of IL-22mAb also decreased the levels of proinflammatory cytokines, but they were still higher than the controls. This suggests that although Th22 cells enhance the secretion of pro-inflammatory factors by HaCaT cells, this enhancement did not exclusively depend on the increase of IL-22.

HRMC cells generally responded similarly to the same factors (Fig. 3f-j). However, HRMC cells had no significant difference in proliferation when the target cells had contact or no contact, but they had a higher IFN- α level when the cells had contact than no contact.

Th22 stimulates HaCaT and HRMC cells by activating PI3K/AKT signaling

Based on the optimal E:T cell ratio of 4:1, we examined the effect of co-culturing of target cells with Th22 cells on the expression of proteins in the PI3K/AKT signaling pathway (Fig. 4). The results indicated that this treatment led to significant upregulation of AKT, p-AKT (Thr308), and p-AKT (Ser473) in HaCaT cells and HRMC cells. Treatment with 30 μ M LY294002 (a PI3K inhibitor) or IL-22mAb reduced this effect, and LY294002 had a stronger effect than IL-22mAb.

We further examined the role of PI3K/AKT signaling by studying its role in cell proliferation and cytokine secretion. The proliferation of HaCaT cells (Fig. 5a) was significantly greater when they were co-cultured with Th22 cells (Fig. 5a), as were the levels of proinflammatory cytokines (Fig. 5b, c, d). Addition of 30 μ M LY294002 remarkably decreased these effects, indicating the Th22 cells function via the PI3K/AKT signaling pathway; however, the level of TNF- α was still higher than the control, suggesting that other pathways may also promote the secretion of proinflammatory cytokines. IL-22mAb also downregulated cell proliferation, but its effect was much weaker. IL-22mAb also significantly downregulated the cytokine levels, but their levels were still higher than the controls. IL-22mAb also had a weaker suppression of IL-6 secretion.

HRMC cells generally responded similarly to the same factors (Fig. 5E-H).

Discussion

Recent studies have documented that Th22 cells contribute to the pathology of several autoimmune diseases. However, few studies have examined the roles of Th22 cells in the pathogenesis of SLE, especially the effects of these cells on their target cells. Skin and kidney involvement are common in SLE patients. Our previous study reported that Th22 cells targeted mesangial cells of the kidney and basal keratinocytes of the skin(33). The present study examined the potential roles and the mechanism of Th22 cells in promoting cell proliferation and secretion of proinflammatory cytokines by target cells.

Our results demonstrated increased levels of IL-22 when Th22 cells were co-cultured with target cells (HaCaT or HRMC cells). The proliferation of target cells increased when they were co-cultured with Th22 cells and when rIL-22 was added to the culture, and the proliferation was greater when the cells had direct contact during co-culturing. Co-culturing of target cells with Th22 cells also increased the levels of three pro-inflammatory cytokines (IL-6, TNF- α , and IFN- α). Furthermore, addition of IL-22mAb completely blocked the effect of IL-22 on cell proliferation; addition of IL-22mAb also decreased the levels of pro-inflammatory cytokines, although they were still higher than the controls.

Th22 cells express a variety of skin-homing receptors, including CCR6, CCR4, and CCR10(6, 34). These receptors mediate the infiltration of Th22 cells into skin tissues, suggesting that they function in skin damage. Previous studies reported the role of Th22 cells and IL-22 in psoriasis and other skin diseases, such as allergic dermatitis and scleroderma(35, 36). Our previous study found that SLE patients with skin involvement had higher levels of Th22 cells than those without skin involvement. Additional observations using immunofluorescence staining and confocal microscopy indicated that the expression of IL-22R1

(an IL-22 receptor) on keratinocytes, and that Th22 cells infiltrated the skin around keratinocytes(33). Taken together, these data suggest that Th22 and IL-22 target keratinocytes.

Keratinocytes function as immunomodulators, in that they secrete inhibitory cytokines, stimulate inflammation, and activate Langerhans cells in response to injury(37). Keratinocytes may also be targeted in several chronic inflammatory systemic diseases. When there is an autoimmune attack against the attachment proteins of keratinocytes, they undergo apoptosis and stimulate several subsequent pathological events. For example, in psoriasis, activated T lymphocytes release various cytokines to stimulate the proliferation of keratinocytes(38). Cytokine-activated keratinocytes can also express a broad array of cytokines, chemokines, and membrane molecules that induce the recruitment and activation of T lymphocytes in the skin(39–41). A previous study demonstrated the role of keratinocytes in cutaneous lupus erythematosus (CLE) or SLE with skin involvement(42). A previous study showed that apoptosis of keratinocytes occurred rapidly following exposure to ultraviolet radiation (UVR) and that photosensitivity was associated with increased apoptosis of these cells(43). Apoptotic keratinocytes can also express autoantigens that bind to autoantibodies, leading to complement activation and sustained skin inflammation(42). These many studies thus demonstrate that keratinocytes function as targets and active participants in the inflammatory process.

The presence of lupus nephritis (LN) in patients with SLE is associated with poor prognosis and increased risk of death, possibly because IL-22 targets the kidney. In agreement with this interpretation, a previous study found that IL-22 induced the production of complement proteins, indicating that it may also indirectly affect glomerular epithelial cells through complement and participate in the pathogenesis of LN(44). A recent study by Yang et al. demonstrated increased IL-22 levels in the blood and kidney tissues of LN patients and MRL/lpr mice, and that blocking of IL-22 with anti-IL-22mAb inhibited the progression of LN in the mouse model(11). Our previous study found expression of IL-22R1 in mesangial cells of the renal glomeruli of LN patients, suggesting that IL-22 may target renal mesangial cells(33). Similarly, Lu et al. found that renal mesangial cells and renal tubular epithelial cells induced the infiltration of Th22 cells(45). Renal mesangial cells have an important function in the glomerulus, and they can interact with immune deposits, complement components, and inflammatory mediators(46). The proliferation of mesangial cells and the excessive production of mesangial matrix are typical manifestations of renal biopsies in patients with active LN(47).

We further studied the effect of Th22 cells on keratinocytes and renal mesangial cells by performing in vitro experiments with HRMC and HaCaT cells. In particular, we used the Transwell system to co-culture these target cells with Th22 cells or treat them with rIL-22. Our results showed that IL-22 promoted the proliferation of these target cells and their production of pro-inflammatory cytokines. Consistent with our present results, previous studies suggested that IL-22 promoted the proliferation of multiple epithelial and mesenchymal cells, such as keratinocytes, renal mesangial cells, synovial fibroblasts, and intestinal epithelial cells(48). To maintain a homeostatic balance, these pathologically proliferated cells underwent apoptosis. Due to the reduced function of apoptotic clearance in SLE, necrosis and local inflammation worsens, and this may activate the immune system to produce autoantibodies and immune complexes,

causing systemic damage(49). Other research showed that IL-22 promoted the production of IL-1, IL-6, IL-18, and other proinflammatory cytokines by skin cells exposed to UVR(43). Another study demonstrated that Th22 cells promoted the production of IL-6 and TNF- α by HRMC cells when co-cultured together with contact(45). We also found that Th22 cells induced the proliferation and secretion of pro-inflammatory cytokines in HaCaT and HRMC cells when they were co-cultured. Co-culturing of these cells also increased the level of IL-22, thus indicating that Th22 cells act upon their target cells via the production of IL-22. When Th22 cells were co-cultured in contact with the target cells (rather co-cultured without contact), this further increased the production of IL-22 and cell proliferation.

Our results suggest that Th22 cells promote the proliferation of HaCaT cells by secreting of IL-22 and by physical contact during co-culture. In contrast, Th22 cells had similar effects on HRMC cells during co-culture with and without physical contact. This difference is intriguing and suggests the need for further research on additional cell lines. We also found that IL-22mAb completely blocked the effect of IL-22, in that it prevented cell proliferation, suggesting that the promotion of target cell proliferation depended on IL-22. IL-22mAb significantly decreased the levels of pro-inflammatory cytokines (IL-6, TNF- α , and IFN- α), but their levels remained higher than the controls. This suggests that although Th22 cells enhance the secretion of pro-inflammatory cytokines by target cells, other factors are also important. Considering all these results together, we speculate that during the pathogenesis of SLE, Th22 cells infiltrate their target cells and have full physical contact with them via cytokine receptor-ligand interactions, and that this leads to enhanced secretion of IL-22, thereby promoting disease progression.

The binding of IL-22 to IL22R leads to activation of JAK1 (mobile kinase IL22R1) and Tyk2 (mobile kinase IL10R2), which trigger multiple intracellular pathways, such as MAPK, AKT, P38, JNK, and ERK1/2(23). The PI3K/AKT pathway has links with many intracellular signaling pathways, and functions in the regulation of various cellular events, including cell proliferation, angiogenesis, invasion, and metastasis. Our results indicated that Th22 cells markedly activated PI3K/AKT signaling in vitro. Other studies reported similar molecular effects in different diseases, such as UVB-induced skin inflammation and experimental autoimmune uveitis(22, 50–52). To confirm the role of PI3K/AKT signaling, we assessed the effects of separate additions of LY294002 and IL-22mAb. The results indicated that LY294002 downregulated the protein levels of AKT, p-AKT(Thr308), p-AKT(Ser473), and also downregulated the secretion of IL-6, TNF- α , and IFN- α . LY294001 also blocked the proliferation of co-cultured HaCaT and HRMC cells; IL-22mAb had a similar but weaker suppressive effect. These results suggest that PI3K/AKT signaling functions in target cell-mediated proliferation and secretion of pro-inflammatory cytokines. Thus, inhibition of this pathway using LY294001, IL-22mAb, or another agent may have potential for the therapy of SLE.

In summary, our findings provide a preliminary description of the potential mechanism by which Th22 cells interact with their target cells – keratinocytes and renal mesangial cells. We also identified a possible role of PI3K/AKT signaling in SLE, and IL-22 as a possible therapeutic target for the treatment of SLE. Our future studies will examine the effects of different interventions involving Th22 cells and IL-22 in animal models. We will also investigate other T cell subsets, such as T_{FH} and Treg cells, to examine

organ involvement in SLE by using a large sample size and performing assessments at multiple times during disease progression.

Materials And Methods

Target cell lines

Two lines of target cells, HRMC and HaCaT cells (ScienCell, San Diego, CA), were maintained in RPMI-1640 medium or DMEM (HyClone, Logan, QLD) that contained 10% heat-inactivated FBS (Gibco, New York, NY), penicillin (100 U/mL), and streptomycin (100 U/mL) (HyClone). Cells were incubated in humidified air with 5% CO₂ at 37 °C and subcultured with 0.25% trypsin and 0.02% EDTA.

Isolation of human peripheral blood mononuclear cells

Five healthy donors were recruited from August 2018 to October 2018 from the Central Blood Bank, Changchun, China. After overnight fasting, each participant was subjected to a peripheral blood draw of 40 mL. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation with Ficoll-Paque plus density gradient media (GE Healthcare Bio-Sciences, Pittsburgh, PA). PBMCs (1×10^7 /mL) were maintained in RPMI-1640 medium that was supplemented with 10% heat-inactivated FBS (Gibco) and stored at 4 °C before subsequent procedures.

Th22 cell separation and purification by flow cytometry

PBMCs were resuspended in HBSS (without calcium or magnesium, but supplemented with 2% heat-inactivated FBS) and stained in duplicate in the dark at 4 °C for 30 min with the following antibodies (all from Becton Dickinson, San Diego, CA): APC-H7-anti-CD4, BV510-anti-CCR4, PE-Cy7-anti-CCR6, and BB515-anti-CCR10. Negative controls were stained with isotype-matched control antibodies (APC-H7-anti-IgG1, BV510-anti-IgG1, PE-Cy7-anti-IgG1, and BB515-anti-IgG2a). Then, CD4 + CCR6 + CCR4 + CCR10 + Th22 cells were obtained after separation and purification by flow cytometry. The resulting cells (concentration: 1×10^5 /mL, purity > 95%) were used in experiments (Fig. 6).

Co-culture of Th22 cells with target cells

The cultured target cells were collected when the confluence was 90%. After transfer to 96-well plates (3×10^3 /200 μ L/well), the target cells were cultured in an incubator (37 °C and 5% CO₂) until they were in the logarithmic growth stage. The supernatant was discarded, and 200 μ L of effector Th22 cells and Dynabeads Human T-Activator (1:1 ratio) were added to each well. The ratios of Th22 to target cells were 0:1, 0.5:1, 1:1, 2.5:1 and 4:1 (corresponding to 0, 1500, 3000, 7500, and 12000 Th22 cells per well). The cells were then co-cultured in RPMI-1640 medium with 10% heat-inactivated FBS (Gibco), 100 U/mL penicillin, 100 g/mL streptomycin, 50 g/mL gentamicin, 2 mM L-glutamine, and 10 mM HEPES in an incubator (37 °C and 5% CO₂). After 48 h, the supernatant was collected for determination of cytokine concentrations using an enzyme-linked immunosorbent assay (ELISA, see below). Then 100 μ L of culture

medium was added to each well, and the cell proliferation was determined using the Cell Counting Kit-8 (CCK-8, see below).

Co-culture of IL-22 with target cells

As above, instead of Th22 cells, 200 μL of different concentrations of rIL-22 (0, 10, 30, 100 ng/mL; R&D System, Minneapolis, Minnesota) were added to each well of a 96-well plate that had target cells ($3 \times 10^3/200 \mu\text{L}/\text{well}$). After 48 h, the supernatant was collected and cytokines were determined using ELISA. Then, 100 μL of culture medium was added to each well, and the cell proliferation was determined using the CCK-8 kit.

Transwell co-culture systems

The CD4 + CCR6 + CCR4 + CCR10 + Th22 cells (purity > 95%) were obtained by flow cytometry as described above. Cultured target cells during the logarithmic growth phase were added to bottom of the lower chamber of a Transwell (1×10^4 cells/well). Different amounts of Th22 cells (according to the optimal effector:target [E:T] ratio selected in this study) were added to the upper (non-contact group) or lower (contact group) chambers. The Transwell co-culture systems were established as follows.

Group A, target cells alone.

Group B, negative control (NC). Dynabeads Human T-Activator (same amount as other groups) in the lower chamber. Co-cultured with target cells.

Group C, contact group. Effector Th22 cells and Dynabeads Human T-Activator (1:1 ratio) in the lower chamber. Co-cultured with target cells.

Group D, non-contact group. Effector Th22 cells and Dynabeads Human T-Activator (1:1 ratio) in the upper chamber. Co-cultured with target cells.

Group E, contact + IL-22mAb group. Effector Th22 cells, Dynabeads Human T-Activator and Th22 cells (1:1 ratio), and 10 $\mu\text{g}/\text{mL}$ IL-22mAb (R&D System, Minnesota) in the lower chamber. Co-cultured with target cells.

Group F, non-contact + IL-22mAb group. Effector Th22 cells, Dynabeads Human T-Activator and Th22 cells (1:1 ratio) and 10 $\mu\text{g}/\text{mL}$ IL-22mAb (R&D System) in the upper chamber. Co-cultured with target cells.

Group G, rIL-22 group. Dynabeads Human T-Activator (the same amount as other groups) and rIL-22 (R&D System, Minnesota), according to the optimal concentration selected in this study, in the lower chamber. Co-cultured with target cells.

After 48 h, the supernatant was collected to determine the cytokine concentrations using ELISA. The target cells were counted and transferred to 96-well plates ($3 \times 10^3/200 \mu\text{L}/\text{well}$) with 100 μL culture

medium in each well. The cell proliferation was determined using the CCK-8 kit.

Detection of cytokine concentrations by ELISA

The concentrations of plasma IL-22, IL-6, TNF- α , and IFN- α in each well were determined by ELISA using specific cytokine kits, according to the manufacturer's protocols (eBioscience, San Diego, CA).

CCK-8

Cell counts were determined using the CCK-8 kit (Dojindo, Kumamoto). The detection reagent (10 μ L) was added to each well, and absorbance (OD_{450nm}) was measured after 2 h of incubation.

Western blotting

Target cells were harvested in a RIPA buffer (Thermo Fisher Scientific, Waltham, MA) with 1% PMSF on ice. Protein concentration was determined using the bicinchoninic acid assay (Sigma, Saint Louis, MO) following the manufacturer's instructions. Polyclonal primary antibodies (anti-Akt, BD Biosciences, Bergen County, NJ; anti-phosphorylated Akt Ser473, Cell Signaling Technology, Danvers, MA; and anti-phosphorylated Akt Thr308, Cell Signaling Technology) were used for immunoblotting. Anti-actin (Sigma) was routinely used as a protein loading control. The secondary antibody was anti-rabbit HRP-IgG (Amersham, Piscataway, NJ). An enhanced chemiluminescence assay (Amersham) was used for visualization following the manufacturer's instructions. Image J software was used to analyze gray-scale levels. The average ratio of the target protein to the internal reference (actin) was used for semiquantitative analysis.

Statistical analysis

Data are expressed as means \pm SEMs of 3 independent experiments. The t-test was used for comparisons of different groups. GraphPad Prism 6 software was used for data analysis and production of graphics. A two-sided p value below 0.05 was considered significant.

Declarations

Ethics approval and consent to participate

All experimental procedures involving human samples were conducted with strict adherence to the guidelines of the Declaration of Helsinki. The study protocol was approved by the Human Ethics Committee of Jilin University, and each subject provided written informed consent.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Zhuang Ye designed the whole research. Ling Zhao performed the most of immunology experiments. Dejun Sun and Miaonan Sun collected human blood samples. Zhenyu Jiang organized the data.

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Figures

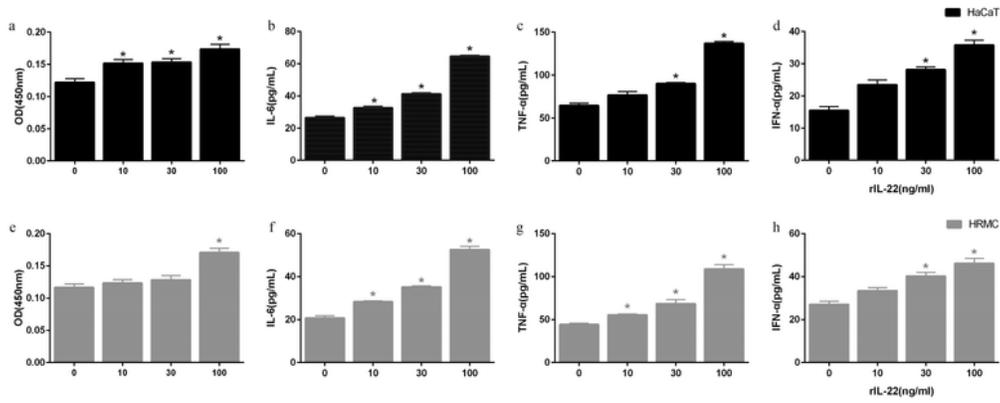


Figure 1

Effect of rIL-22 concentration on the proliferation of HaCaT cells (A) and HRMC cells (E) and the production of three pro-inflammatory cytokines by these cells (B, C, D and F, G, H). Data are presented as means \pm SEMs. *P<0.05 compared with the control.

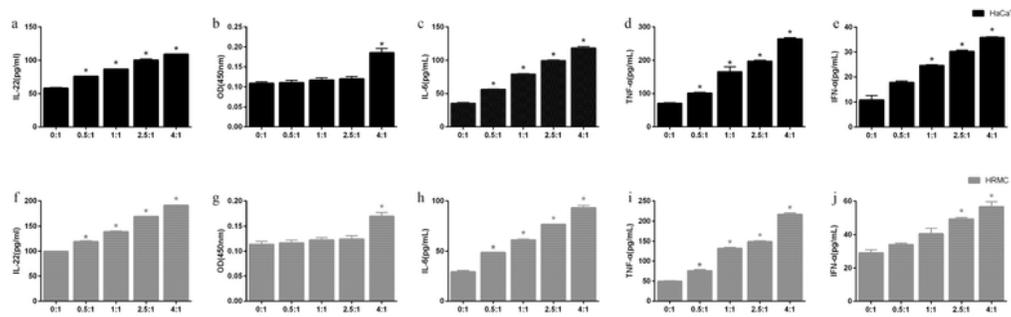


Figure 2

Effect of different cell ratios on the IL-22 levels, cell proliferation, and production of pro-inflammatory cytokines during co-culture of Th22 cells with HaCaT cells (top) and HRMC cells (bottom). A and F: IL-22 secretion. B and G: Target cell proliferation induced by different cell ratios. C, D, E and H, I, J: Levels of three pro-inflammatory cytokines in the supernatants. Data are presented as means \pm SEMs. *P<0.05 compared with the 0:1 group (control).

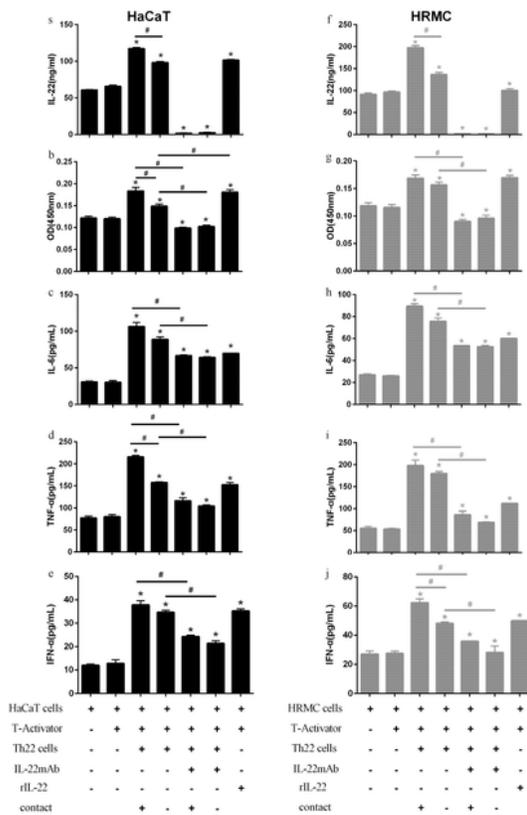


Figure 3

Effect of IL-22 on cell proliferation and production of pro-inflammatory cytokines by HaCaAT cells (left) and HRMC cells (right) in the Transwell co-culture system. A and F: IL-22 levels. B and G: Target cell proliferation. C, D, E, and H, I, J: Levels of pro-inflammatory cytokines in the supernatants. Results are presented as means \pm SEMs. *P<0.05 comparison with the T-Activator+target cells (NC) group, #P<0.05 comparison of the two indicated groups.

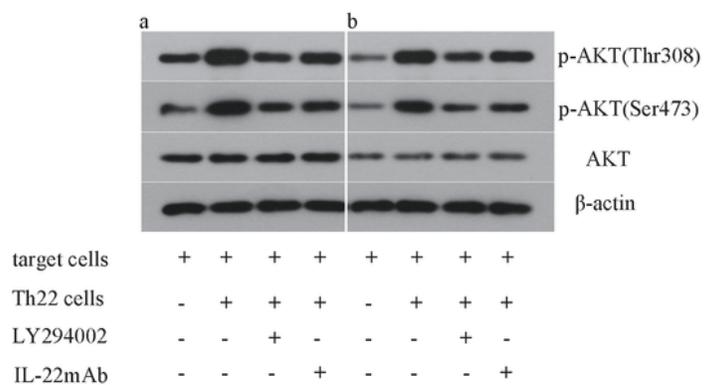


Figure 4

Western blot analysis of the levels of p-AKT (Thr308), p-AKT (Ser473), and AKT in HaCaT cells (a) and HRMC cells (b) after different treatments.

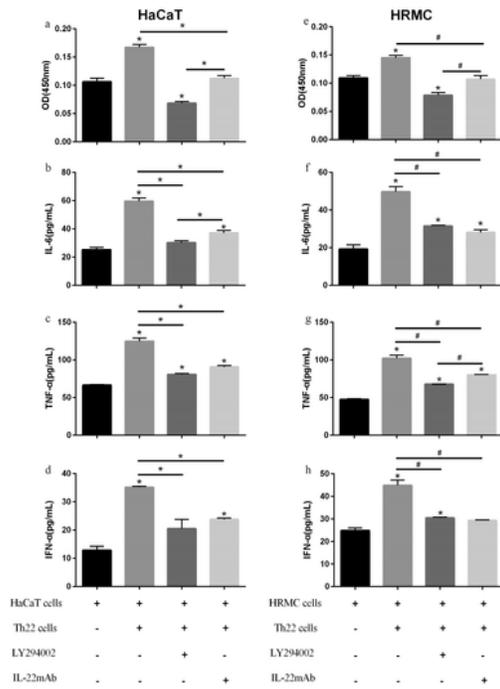


Figure 5

Cell proliferation (A and E) and production of pro-inflammatory cytokines (B,C,D and F, G, H) after different treatments of HaCaT cells (left) and MRMC cells (right). Results are presented as means \pm SEMs. *P<0.05 comparison with the control group. #P<0.05 comparison of the two indicated groups.

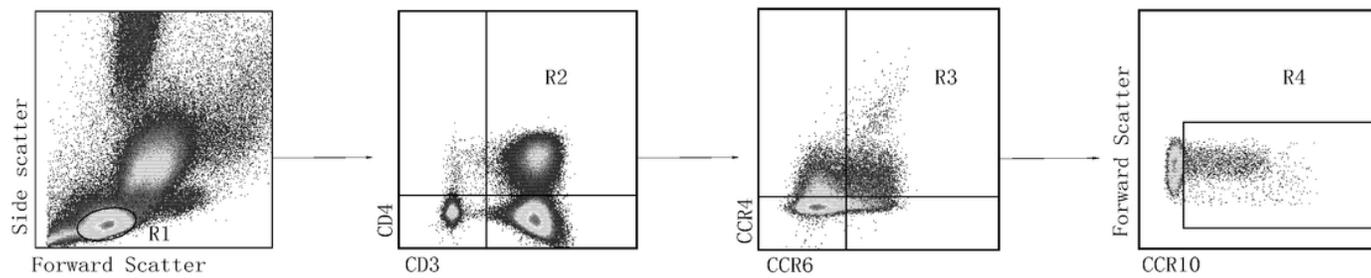


Figure 6

FACS gating strategy used for Th22 cell sorting.