Are Peripheral Natural Killer Cells and Interleukin-21 Interrelated in Psoriasis Pathogenesis?

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Dear Editor:
Psoriasis is a genetically programmed disease of dysregulated inflammation, which is initiated and maintained by pathologic collaboration between innate and acquired immunity resulting in the production of cytokines, chemokines, and growth factors.

Interleukin-21 (IL-21) is an immunomodulatory cytokine with pleiotropic effects on both innate and adaptive immune responses, and is produced by natural killer (NK) T cells, CD4⁺ T cells, and T helper 17 cells. IL-21 actions include positive effects as enhanced proliferation of lymphoid cells, and increased cytotoxicity of CD8⁺ T cells and NK cells, and negative effects as direct inhibitory action on the antigen-presenting function of dendritic cells.

NK cells (CD3⁻CD56⁺) are innate immune effectors that play a pivotal role in the anti-inflammatory response and tumor surveillance, and in addition they are newly identified protagonists in the pathogenesis of psoriasis with distinctive homing properties to psoriatic skin, particularly the CD56brightCD16⁻ cell subset. IL-21 was found to have multiple effects on NK cells development and function.

The present work aimed to investigate the changes in serum IL-21 level and in the frequency of peripheral NK cell phenotype subsets in psoriasis patients in comparison to healthy subjects, and to relate them to each other, and to disease severity in a trial to elucidate the role and inter-relation of these immune effectors in the pathogenesis and severity of psoriasis.

Thirty untreated psoriasis patients and twenty age and sex-matched healthy controls were included. Psoriasis severity was assessed by psoriasis area and severity index (PASI). Serum IL-21 was assessed by human IL-21 Platinum ELISA (eBioscience Inc., San Diego, CA, USA), while immunophenotyping and evaluation of CD3, CD56 and CD16 in peripheral blood lymphocytes were done using Becton Dickinson (BD) fluorescence activated cell sorter (FACS) (Calibur Flow Cytometer, San Jose, CA, USA). FACS Calibur Flow Cytometry from BD was used for analysis, and automated CellQuest Pro software was used for data acquisition and analysis. This study was approved by the Research Ethics Committee of the Faculty of Medicine-Tanta University (No. 1309/07/14).

The clinical characteristics, serum IL-21 levels, and peripheral NK cell populations of studied psoriasis patients in comparison to healthy controls are summarized in Table 1. There was a statistically significant increase in serum level of IL-21 in psoriasis patients compared to controls (p=0.001). Serum level of IL-21 showed a significant positive correlation with PASI score (r=0.793, p=0.001). This increase in serum level of IL-21 in patients with severe psoriasis could be attributed to the more pronounced inflammatory cell milieu which is the source of IL-21 production. This higher level of IL-21 could share in initiating and augmenting the inflammation and epidermal hyperplasia which were reflected as an increase in psoriasis severity. Sarra et al. confirmed a reduced epidermal thickness and reduced inflammatory cell numbers in xenograft from mice treated with IL-21-specific antibody. They stated that IL-21 might play an important role in psoriatic epidermal hyperplasia, parakeratosis, and inflammatory infiltration.

In the current study, flow cytometric analysis revealed sig-
Table 1. Patients’ profiles in comparison to healthy controls

| Variable                                      | Psoriasis patients (n=30) | Control (n=20) | p-value   |
|-----------------------------------------------|---------------------------|----------------|-----------|
| Age (yr)                                      | 35.07±6.50 (18~44)        | 31.20±7.02 (21~41) | 0.056†    |
| Sex                                           |                           |                |           |
| Male                                          | 19 (63.3)                 | 13 (65.0)      | 0.574‡    |
| Female                                        | 11 (36.7)                 | 7 (35.0)       |           |
| Disease duration (mo)                         | 53.53±54.48 (1~240)       | NA             |           |
| Family history                                |                           |                |           |
| Positive                                      | 11 (36.7)                 | NA             |           |
| Negative                                      | 19 (63.3)                 | NA             |           |
| Psoriasis severity according to PASI score    |                           |                |           |
| Mild                                          | 11 (36.7)                 | NA             |           |
| Moderate                                      | 10 (33.3)                 | NA             |           |
| Severe                                        | 9 (30.0)                  | NA             |           |
| Serum IL-21 (pg/ml)                           | 87.2±34.96 (45~190)       | 42.8±8.55 (30~60) | 0.001*†   |
| Percentage of peripheral NK cell subsets      |                           |                |           |
| % of CD3-CD56<sup>high</sup>CD16<sup>-</sup>   | 1.47±0.65 (0.5~2.2)       | 2.35±0.7 (1.1~4.8) | 0.001*†   |
| % of CD3-CD56<sup>high</sup>CD16<sup>+</sup>   | 0.096±0.025 (0.04~0.1)    | 0.15±0.044 (0.06~0.2) | 0.381†    |
| % of CD3-CD56<sup>dim</sup>CD16<sup>-</sup>    | 7.15±1.19 (5~9)           | 8.89±0.67 (7.7~9.9) | 0.001*†   |
| % of CD3-CD56<sup>dim</sup>CD16<sup>+</sup>   | 0.071±0.021 (0.034~0.1)   | 0.117±0.035 (0.06~1.09) | 0.001*†   |

Values are presented as mean±standard deviation (range) or number (%).
PASI: psoriasis area and severity index, IL: interleukin, NK: natural killer, NA: not applicable.
*Significant, †according to Student t-test, ‡according to chi-square test.

Fig. 1. (A) Light side scatter analysis (SSC) of peripheral blood with a gate encompassing the CD3<sup>-</sup> (non T-cell) lymphocyte population (R2). (B) Flow cytometry dot blot analysis on peripheral blood lymphocytes isolated from a psoriatic patient showing different natural killer (NK) cell subsets according to CD56/CD16 positivity; CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>-</sup> (R3), CD3<sup>-</sup>CD56<sup>dim</sup>CD16<sup>-</sup> (R4), CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>-</sup> (R5), and CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>+</sup> (R6) peripheral NK cell subsets. PerCP: peridinin-chlorophyll-protein complex, FITC: fluorescein isothiocyanate, PE: phycoerythrin.

Significantly fewer peripheral NK cells with CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>-</sup>, CD3<sup>-</sup>CD56<sup>dim</sup>CD16<sup>-</sup>, CD3<sup>-</sup>CD56<sup>dim</sup>CD16<sup>+</sup>, and CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>-</sup> phenotypes in psoriasis patients compared to controls (all p=0.001; Fig. 1). Consistent peripheral NK cell reductions were previously reported and attributed to shortened NK cell survival or keratinocyte-secreted chemokines (CXCL10, CCL5, and CCL20) that recruit peripheral NK cells to inflamed psoriatic skin<sup>3,6</sup>. Indeed, receptors for these chemokines were identified in high levels on NK cells infiltrating psoriatic skin.<sup>3</sup>

Of note, we detected a significant positive correlation between the frequency of CD3<sup>-</sup>CD56<sup>high</sup>CD16<sup>-</sup>, CD3<sup>-</sup>CD56<sup>dim</sup>CD16<sup>-</sup>, and CD3<sup>-</sup>CD56<sup>dim</sup>CD16<sup>+</sup> NK cell subsets in psoriatic patients and psoriasis disease duration (p=0.027, 0.045, 0.001 respectively) which could be due to early inflammatory cell and cytokine surges and their
impact on NK cell biology. A previous study described an up-regulation of FAS receptor (FasR) on NK cells of new-onset psoriasis patients which could render these cells more prone to apoptosis. It should be noted that IL-21 is a pro-apoptotic factor for NK cells, but the combination of IL-15 diminishes this IL-21-mediated effect. Moreover, we detected a statistically significant negative correlation between the CD3 \(^{-}\) CD56\(^{bright}\)CD16\(^{-}\) NK cell population and PASI score (\(r=-0.351\), \(p=0.042\)). On the contrary, no significant correlation was found between CD3 \(^{-}\) CD56\(^{bright}\)CD16\(^{+}\), CD3 \(^{-}\) CD56\(^{dim}\) CD16\(^{+}\), or CD3 \(^{-}\) CD56\(^{dim}\) CD16\(^{-}\) NK cell populations and PASI (\(r=-0.047\), \(p=0.81\); \(r=-0.154\), \(p=0.42\); \(r=-0.236\), \(p=0.22\) respectively). Our findings could be explained by that CD3 \(^{-}\) CD56\(^{bright}\)CD16\(^{-}\) cells represent the activated, cytokine-production efficient NK cell population, and so they could be more severely recruited to lesional psoriatic plaques in patients with higher PASI, and their cytokine products might add to inflammation severity and keratinocyte proliferation.

In this study, none of the changes of peripheral NK cell subsets showed any statistically significant correlation with serum level of IL-21 (all \(p>0.05\)). As the effect of IL-21 on NK cell biology is sophisticated depending not only on its concentrations, but also on presence of other cytokines, these complex interactions could explain the detected absence of significant direct correlation between changes in peripheral NK cell populations and serum IL-21 levels in psoriatic patients in our study. For example, when IL-21 is combined with IL-7, IL-15, and stem cell factor, it augments the generation of NK cells in vitro. Moreover IL-21 can augment the cytolytic activity of NK cell, with IL-21-induced expression of killer inhibitory receptors on these cells. IL-21 can act synergistically with IL-15 to augment the production of IFN- \(\gamma\) and granzyme-B by NK cells. IL-21 can augment proliferation of NK cells, and its low concentrations are stimulatory whereas higher doses are often inhibitory.

Collectively speaking, the manifest alterations detected in this work in serum IL-21 level and peripheral NK cell population in psoriasis suggest their important roles in the complex web of psoriasis pathogenesis. Cross-talks between them are thought to be multifactorial, but not clearly established yet. Our study might provide insight into application of serum IL-21 and peripheral percentage of CD3 \(^{-}\) CD56\(^{bright}\)CD16\(^{-}\) NK subset as predictors of psoriasis disease severity. Moreover, targeting IL-21 might be a potential immunotherapeutic hope for psoriasis. Further work is needed to study the use of NK cell modulation therapy in psoriasis.

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