Is neutrophil the dominant IL-17 producer in psoriasis?

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Figure 1. (a) Neutrophils purified by density gradient centrifugation were analyzed by flow cytometry (monocyte is CD14⁺ high; blue, neutrophil is CD14⁺ low; green, CD3⁺ T cell is red). (b) The purity of the neutrophils collected by a specific magnetic beads separation shows remarkable improvement in samples from normal control and severe psoriasis patient. (c) mRNA of IL-17A and GAPDH was amplified by RT-PCR. Lanes 1 and 10 were size markers. FSC, forward scatter; SSC, side scatter.

Introduction

- Neutrophil is the majority in the psoriatic lesions, and have been suggested IL-17 producer, however, the real IL-17 supplier in psoriasis is still controversial.
- Presence of IL-17 immuno-reactive neutrophils in the psoriatic skin lesions has been reported histopathologically as well as in peripheral blood by flow cytometric analysis.¹
- A report declared absence of IL-17 mRNA expression by reverse transcription (RT) -PCR,² others reported presence in the severe psoriasis patients.
- Is there any technical issue to address this inconsistency?
- Here, we report that the difference of two method of neutrophil separation and IL-17 may not be produced from neutrophils.

Material and Methods

Flow cytometry

- Whole blood was collected from psoriasis patient and healthy control into heparinized blood collect tubes.
- Neutrophils, monocytes and lymphocytes were isolated by density gradient centrifugation with Histopaque®-1077 and Histopaque®-1119.
- Neutrophil was also isolated by specific magnetic bead separation from whole blood.
- The collected cells were stained with fluorescein isothiocyanate (FITC) conjugated anti-CD14 antibody and PerCp-conjugated anti-CD3 antibody and analyzed by flow cytometry.

RT-PCR

- Peripheral blood mononuclear cells (PBMC) isolated by Histopaque®-1077 were purified by monocytes or CD3⁺ T cells specific magnetic bead separation.
- mRNA was extracted from collected cells by ISOGEN-LS®.
- The primer for RT-PCR were IL-17A and internal control glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Reference

- Lin AM, et al. *J Immunol* 2011; 187: 490–500.
- Tamarozzi F, et al. *Immunol Lett* 2014; 162: 194–198.
- Yamanaka K, et al. *J Dermatol* 2014; 41: 679–685.

Result

- Neutrophils from a severe psoriasis patient purified by density gradient centrifugation showed merged zone between neutrophils, monocytes and CD3⁺ T cells (Fig. 1a).
- Neutrophils separated by specific magnetic bead separation shows high purity both healthy control and psoriasis patient (Fig. 1b).
- Neutrophil fraction purified by density gradient centrifugation expressed IL-17A mRNA amplification (lanes 2 and 6), however, no obvious band was detected in the neutrophil fraction separated by specific bead separation (lanes 3 and 7). (Fig. 1c)

Conclusion

- In severe psoriasis patients, merged zones among the neutrophils, mononuclear cell and T cell boundary were shown, resulting in low purity in neutrophil fraction.
- Our previous report declared activation of the circulating neutrophils and monocytes in psoriasis⁵, the activated neutrophils are morphologically enlarged with low density.
- The boundaries of each cell population were merged in flow cytometry, and this resulted in mixture of monocytes and lymphocytes with neutrophils. Thus, these aberrant cells may have a role in misunderstanding IL-17A production from neutrophils.
- Neutrophils are not the actual major source of IL-17A in psoriasis, but activated monocytes and lymphocytes are the dominant source of IL-17A. The mixture of monocytes or lymphocyte derived IL-17A mRNA was detected in the neutrophil fraction by RT-PCR.