IL-17A is produced by CD4+ but not CD8+ T cells from synovial fluid (SF) of PsA patients. However, most current studies show normal frequency of Tregs in the PB, but increased in SF, except when stimulated with TCR activation. Similar results were found after 3 days’ co-culture of aCD3 and 28 stimulated CD8+ T cells from SFMCs with autologous PsA CD14+ monocytes. Also, when either co-cultured with autologous CD14+ monocytes, only CD4+ T cells showed mRNA expression of IL-17A and IFNy after 4 hours’ (hrs) phorbol myristate acetate and ionomycin (PMA and iono) stimulation. Blood samples of PsA patients and age/sex-matched healthy volunteers were included as controls for PsA SF. In addition, the rest of SF cells underwent density gradient separation and mononuclear cells (SFMCs) were sorted to use. CD4+ and CD8+ T cells were sorted from SFMCs, and ex vivo cultured with soluble anti-CD3/anti-CD28 (aCD3 and 28), PMA and iono or without stimulation for 4 or 72 hours. Furthermore, CD4+ and CD8+ T cells were co-cultured with either allogeneic PsA fibroblast-like synoviocytes (FLS) or autologous PsA CD14+ monocytes with aCD3 and 28 activation for 72 hours. Culture supernatants were tested for IL-17A and CD17A+ T cells were analyzed with intracellular staining or RT-qPCR.

Results Accumulation of IL-17A+ CD8+ T cells was significantly higher in PsA SF compared to in blood of either PsA patients or healthy volunteers. Although both CD4+ and CD8+ T cells from SFMCs are IL-17A+ with flow cytometry, only CD4+ T cells produce measurable amounts of IL-17A in culture supernatants after 72 hours of aCD3 and 28 activation. Similar results were found after 3 days’ co-culture of aCD3 and 28 stimulated CD8+ T cells from PsA FLS. Also, when either co-cultured with autologous CD14+ monocytes, only CD4+ T cells showed mRNA expression of IL-17A and IL-17A production. In contrast, if PMA and iono were used to stimulate CD4+ or CD8+ T cells, both produce IL-17A in supernatants after 4 or 72 hours.

Conclusions Although both CD4+ and CD8+ T cells show IL-17A positivity in PsA SF with flow cytometry, the use of strong stimuli such as PMA and iono during intracellular staining may account for the IL-17A positivity in CD8+ T cells. The contribution of CD8+ T cells to IL-17A production in local PsA joints might be limited as normal TCR activation, mimicked by aCD3 and 28, didn’t induce IL-17A release in CD8+ T cells in contrast to CD4+ T cells.

Disclosure of Interest None declared.

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References

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P103/O14 INTERLEUKIN-17 IS PRODUCED BY CD4+ BUT NOT CD8+ T CELLS AFTER TCR ACTIVATION IN SYNOVIAL FLUID OF PSORIATIC ARTHRITIS PATIENTS

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Introduction Interleukin-17A (IL-17A) contributes to the pathogenesis of psoriatic arthritis (PsA) as evidenced by the success of biologics targeting IL-17 pathway in PsA patients. Both CD4+ and CD8+ T cells are reportedly producers of IL-17A in PsA with IL-17A+ CD8+ T cells specifically enriched in PsA synovial fluid (SF). However, most current findings regarding IL-17A-producing cells in PsA patients are coming from intracellular staining using flow cytometry.

Objectives To confirm and compare ex vivo production of IL-17A by CD4+ and CD8+ T cells from PsA synovial fluid using flow cytometry, enzyme-linked immunosorbent assay (ELISA) and RT-qPCR.

Methods Fresh SF of established PsA patients were collected and part of the cells were directly stained intracellularly for IL-17A and IFNy after 4 hours’ (hrs) phorbol myristate acetate and ionomycin (PMA and iono) stimulation. Blood samples of early PsA patients and age/sex-matched healthy volunteers were included as controls for PsA SF. In addition, the rest of SF cells underwent density gradient separation and mononuclear cells (SFMCs) were sorted to use. CD4+ and CD8+ T cells were sorted from SFMCs, and ex vivo cultured with soluble anti-CD3/anti-CD28 (aCD3 and 28), PMA and iono or without stimulation for 4 or 72 hours. Furthermore, CD4+ and CD8+ T cells were co-cultured with either allogeneic PsA fibroblast-like synoviocytes (FLS) or autologous PsA CD14+ monocytes with aCD3 and 28 activation for 72 hours. Culture supernatants were tested for IL-17A and CD17A+ T cells were analyzed with intracellular staining or RT-qPCR.

Results Accumulation of IL-17A+ CD8+ T cells was significantly higher in PsA SF compared to in blood of either PsA patients or healthy volunteers. Although both CD4+ and CD8+ T cells from SFMCs are IL-17A+ with flow cytometry, only CD4+ T cells produce measurable amounts of IL-17A in culture supernatants after 72 hours of aCD3 and 28 activation. Similar results were found after 3 days’ co-culture of aCD3 and 28 stimulated CD8+ T cells from PsA FLS. Also, when either co-cultured with autologous CD14+ monocytes, only CD4+ T cells showed mRNA expression of IL-17A and IL-17A production. In contrast, if PMA and iono were used to stimulate CD4+ or CD8+ T cells, both produce IL-17A in supernatants after 4 or 72 hours.

Conclusions Although both CD4+ and CD8+ T cells show IL-17A positivity in PsA SF with flow cytometry, the use of strong stimuli such as PMA and iono during intracellular staining may account for the IL-17A positivity in CD8+ T cells. The contribution of CD8+ T cells to IL-17A production in local PsA joints might be limited as normal TCR activation, mimicked by aCD3 and 28, didn’t induce IL-17A release in CD8+ T cells in contrast to CD4+ T cells.

Disclosure of Interest None declared.