

LETTER TO THE EDITOR

Atopic dermatitis IL17A- and IFN- γ -producing lymphocytes: investigation in blood, chronic lesions and APT

Editor

Recent findings suggest that an imbalance of Th1, Th2 and Treg plays a critical role in the pathogenesis of AD and that CD8+ T cells are required for the development of AD lesions.^{1,2}

We studied the phenotype of peripheral blood (PB) circulating and intraepithelial lymphocytes deriving from skin affected by chronic moderate–severe AD, acute reaction induced by an atopy patch test to *Dermatophagoides spp* (Dmix/APT) and in skin spared by AD.

After stimulation, skin- and blood-derived mononuclear cells were washed with phosphate-buffered saline and stained with anti-CD4 FITC, anti-CD3 PerCP and anti-CD45 APC antibodies, then fixed with 2% paraformaldehyde and permeabilized with 0.5% saponin/1% fetal calf serum, before an additional staining with intracytoplasmic anti-IL17A PE or anti-IFN- γ PE or control isotype IgG1 PE. Cells were acquired with FACS-CANTO II and analysed with FlowJo software.

Nine patients (8F/1M, mean age 31 years) without antihistaminic, local and/or systemic anti-inflammatory and/or immunosuppressive therapies since ≥ 3 months have been included.

In PB, T cells CD3+ represented the majority of mononuclear cell population and 40–60% of them were CD4+. The phenotypic analysis revealed that in chronic lesions, in skin with acute-Dmix/APT or spared by AD, CD3+CD4+ T lymphocytes were 65%, 67% and less than 60%, respectively, so the amount of CD4+ T cells, differently from CD4– T cells, in both chronic and acute lesions was higher than in skin spared by AD (Fig. 1Aa, Ab).

An average of 2% was the frequency of circulating IL17A-producing T cells with the higher capacity of IL17A production for CD4+ compared to CD4– T cells. A sizeable fraction of both CD4+ and CD4– circulating T subsets physiologically produced IFN- γ , being an average of 25% for CD4+ and more than 30% for CD4– T cells (Fig. 1Ba, Bb).

The frequency of CD4+ IL17A-producing cells was lower in the chronic phase in comparison with skin spared by AD, while there was no difference between this last and in acute phase. A significant difference was observed between chronic and acute lesions (Fig. 2Aa). No statistically significant difference existed

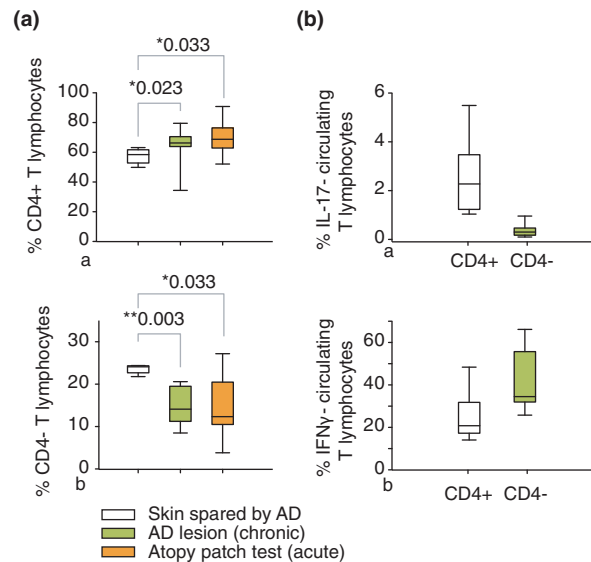


Figure 1 A. Frequencies of CD4+ (Panel Aa) and CD4- (Panel Ab) T, in total CD3+ T, evaluated in AD chronic lesions, atopy patch test (acute reaction) and spared skin of AD patients. B. Frequencies of blood IL17-producing (Panel Ba) and IFN- γ -producing (Panel Bb) cells, in CD4+ and CD4- T.

in the percentages of CD4– IL17A-producing T lymphocytes in acute and chronic phases as well as in skin spared by AD (Fig. 2Ab). Despite that, the results of IFN- γ production indicated a trend of CD4+ and CD4– T lymphocytes from acute lesions to be better IFN- γ producer with respect to those in chronic lesions and skin spared by AD (Fig. 2Ac, Ad).

We found a significant correlation between total CD3+ IFN- γ -producing T lymphocytes from PB and cutaneous biopsy of APT ($P = 0.02$), as well as between CD4+ IFN- γ -producing T lymphocytes between PB and cutaneous biopsy of APT ($P = 0.01$; Fig. 2Ba, Bb).

We can speculate that IL17-A-producing T cells have a role in the surveillance and in the maintenance of the functionality and homeostasis of the immunological barrier by increasing the primary response to exogen pathogens, while subsequently, IL17 levels decrease in chronic phases, characterizing the chronic microenvironment INF- γ -related.^{3–8} Our study showed also that cells secreting IL17 are present in a higher percentage in skin spared by AD and in APT than in PB, most likely for their tissue infiltration, remaining in the skin also in the chronic phase, although at lower frequency.

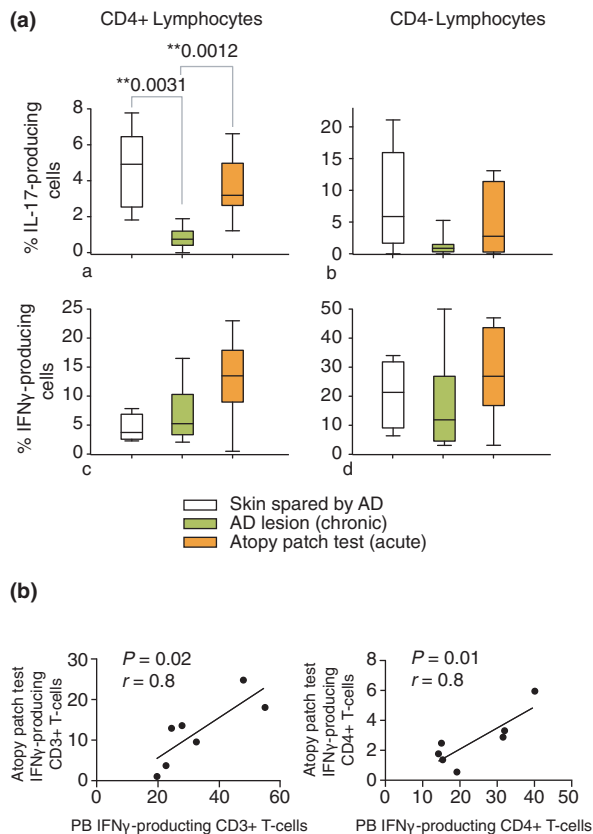


Figure 2 A. Frequencies of IL17-producing (Panel Aa, Ab) and IFN- γ -producing (Panel Ac, Ad) cells, in CD4+ (Panel Aa, Ac) and CD4- (Panel Ab, Ad) T, in AD chronic lesions, atopy patch test (acute reaction) and spared skin. B. Correlation between IFN- γ -producing CD3+ T in blood and APT (Panel Ba) and between IFN- γ -producing CD4+ T in blood and APT (Panel Bb).

We saw a prevalence of CD4+ and CD4- IFN- γ -producing cells in the acute phase, speculating that in this phase, there is also the participation of Th1, while in the chronic phases, CD4+ and CD4- IFN- γ -producing cells seem to decrease. The frequencies of total CD3+ and CD4+ IFN- γ producing T cells found in the acute phase and in PB, positively correlate, so the local immune response in the acute lesions is deeply linked to the individual PB immune profile.

Hijnen *et al.*⁹ found that CD8+ T cells in AD are important producers of IL-13, IL-22 and INF- γ , but not of IL-17. The increased production of IL-13 by CD8+ T cells could play a pivotal role in the destruction of the cutaneous barrier through the suppression of the IL-17 production, the reduction in flaggrin expression and the destruction of tight junctions. These data correlate with our findings of skin- and blood-isolated CD4- T cells being the better IFN- γ producer.

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