Programmed cell death-1-ligand 1 blockade leads to MHC class I dependent IL-17 response in cocultures of CLA+/epidermal cells activated by *Streptococcus pyogenes* in psoriasis

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Introduction

Programmed cell death protein 1 (PD-1) is a co-inhibitory receptor from the CD28 family that mediates the inhibition of the immune system. Blockade of its ligands, PD1-L1 and PD1-L2, constitutes one of the leading immunotherapies against cancer, and it has been reported to induce psoriasis in some patients. The purpose of this study is to address, for the first time, the altered immune response upon PD1/PD1-L1 blockade in psoriasis using a clinically relevant trigger of disease, *Streptococcus pyogenes*, and pathogenic T cells ex vivo.

Materials and methods

The study included 9 psoriatic patients, who previously gave informed consent. Each participant underwent a blood extraction and two skin punch biopsies. Memory CLA+ and CLA- T cells were purified from blood samples through immunomagnetic separations, and epidermal cells (Epi) were obtained by chemical and mechanical treatment of skin punches. $5x10^4$ CLA+ or CLA- T cells were cocultured with $3x10^4$ autologous epidermal cells, and activated by 1μg/ml *Streptococcus pyogenes* extract (SE). CD274 (PD1-L1) and HLA-A/B/C (class I) neutralizing antibodies, or isotype control, were added at day 0, at a final concentration of 10μg/ml. After 5 days of culture, IL-17A and IFN-γ were measured by Diaplex fluorescent bead-based immunoassay, and numbers of CD4+ and CD8+ T cells and their activation (HLA-DR expression) were determined by flow cytometry. Data are represented by scatter plots showing the median (red bar).

Results

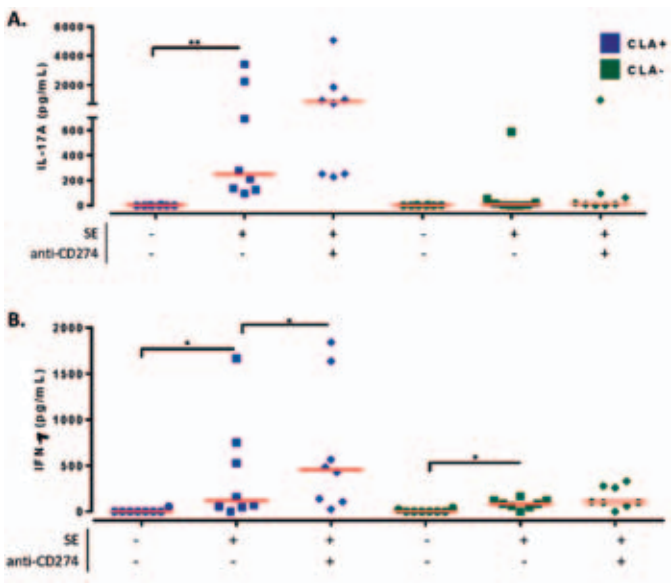

Figure 1. Blockade of CD274 increases CLA+ dependent IL-17A and IFN-γ production after SE stimulation in cocultures. Epidermal cells (Epi) and CLA+ or CLA- T cells from psoriasis patients were cocultured and activated with SE or left untreated, under the presence or not of CD274 neutralizing antibody. Supernatants were collected at day 5 and **(A)** IL-17A (n=8) and **(B)** IFN-γ (n=8) were measured. Paired non parametric t-test was used to compare two different conditions (**: p<0.01, *: p<0.05). After CD274 blockade, an increase in both IL-17A and IFN-? levels after SE stimulation of CLA+/Epi (and not CLA-) cocultures is observed, being notably higher in the case of IL-17A.

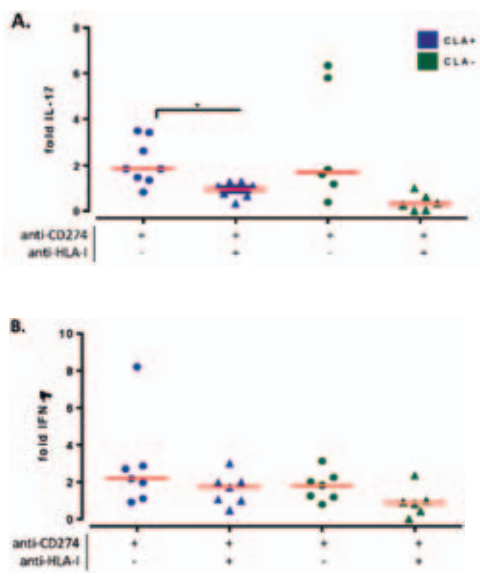

Figure 2. CD274 dependent increase in IL-17A and IFN-γ is HLA class I mediated. We also examined the effect of additionally blocking HLA-I molecules on SE stimulated cocultures, again under the presence or not of neutralizing antibodies against CD274. IL-17 and IFN-γ levels were measured in 5-days supernatants. Ratios of **(A)** IL-17A (n=8) and **(B)** IFN-γ (n=8) levels in each condition against the corresponding isotype control are represented here. Paired non parametric t-test was used to compare two different culture conditions (*: p<0.05). HLA class I blockade reverses the increase of IL-17A production by CLA+ T cells under CD274 blockade, which indicates that HLA-I molecules mediate such increase. Conversely, ratio of IFN-γ levels is slightly affected by HLA-I neutralizing antibody.

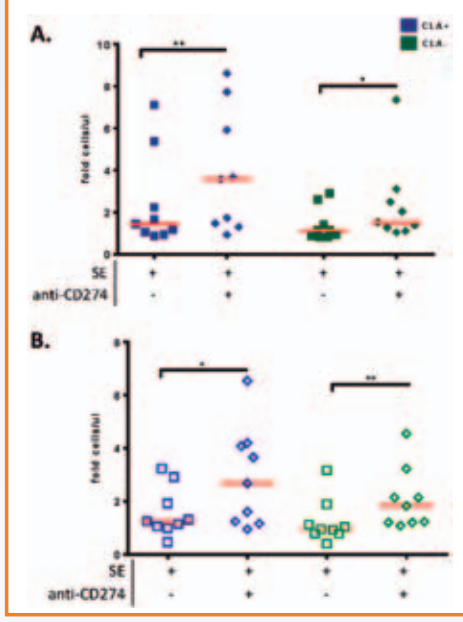

Figure 3. Blockade of CD274 increases the number of T cells upon SE stimulation. Numbers of CD4+ and CD8+ T cells were determined after 5 days of cocultures under the presence or not of CD274 blocking antibody. Ratios of **(A)** CD4+ (n=9) and **(B)** CD8+ (n=9) cells numbers upon SE activation against basal condition (no SE added) are represented. Paired non parametric t-test was used to compare two different culture conditions (**: p<0.01, *: p<0.05). CD274 blockade results in an increase of CD4+ and CD8+ T cell numbers, both in CLA+ and CLA- cultures. Importantly, there is a higher increment in CD4+ CLA+ T cells over CLA- ones. However, CD8+ T cell numbers are similar between CLA+/Epi and CLA-/Epi cocultures.

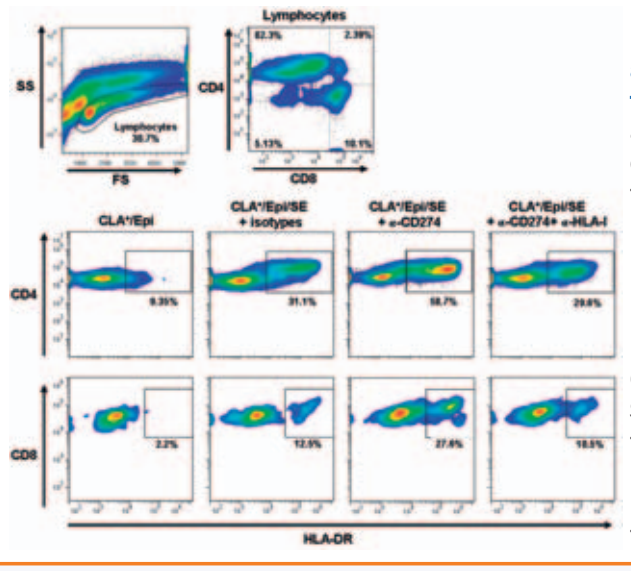

Figure 4. Blockade of CD274 induces the activation of T cells upon SE stimulation through HLA class I molecules. Numbers of activated CD4+ and CD8+ T cells were determined after 5 days of cocultures under the presence or not of CD274 and HLA-I neutralizing antibodies. Activation status was measured by expression of HLA-DR (n=4). A representative patient immune phenotype has been selected to show the results. CD274 blockade increases the percentage of activated CD4+ and CD8+ T cells, when compared to only SE stimulation. And, most interestingly, only the increase of activated CD4+ T cells number is reversed to levels of just SE stimulation when HLA-I neutralizing antibody is added, indicating that HLA-I molecules mediate this process.

Remarks and Conclusions

Altogether, our results show that CD274 blockade induces a significant increase of IL-17A production by CLA+ T cells, as well as it raises CD4+ CLA+ T cell number and activation, and all these mostly depends on HLA class I molecules. These are the first evidences of a connection between IL-17A response and PD1-L1 blockade in a human ex vivo model of psoriasis. In conclusion, our findings might shed new light on the causes behind anti-PD1-induced psoriasis, which could probably be promoted through the HLA class I-dependent generation of activated T cells that produce an exacerbated immune response, mainly via IL-17A cytokine.

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