Staphylococcal Exotoxins Induce Interleukin 22 in Human Th22 Cells

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Key Words

Staphylococcus aureus · Staphylococcal enterotoxin B ·
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Abstract

Background: We have shown previously that T cells from atopic dermatitis (AD) patients produce more IL-22 upon staphylococcal exotoxin stimulation compared to psoriasis patients and healthy controls. The role of staphylococcal exotoxins on polarized memory T helper (Th)22 cells which are enriched in inflamed AD skin remains elusive. Our aim was to investigate IL-22 production in response to staphylococcal enterotoxin B (SEB) and α -toxin stimulation in human memory T cells and polarized Th22 cells. **Methods:** IL-22 induction was investigated in human peripheral blood-derived CD4+CD45RO+CD45RA[−] T cells and polarized Th22 cells after SEB and sublytic α -toxin stimulation in a time-dependent manner at the mRNA and protein (ELISA) levels. **Results:** Th22 cells secreted more IL-22 compared to freshly isolated peripheral blood-derived memory T cells. SEB and α -toxin induced IL-22 in memory T cells as well as in Th22 cells. More IL-22 was induced by SEB and α -toxin in freshly isolated peripheral blood memory T cells compared to Th22 cells derived from memory T cells in long-term cell culture without polarization and Th22 cells under Th22-promoting conditions with IL-6 and TNF- α . No differences in IL-22 induction by staphylococcal exotoxins were observed between

cells from AD compared to psoriasis patients and healthy controls. **Conclusions:** Increased IL-22 secretion can promptly be induced by staphylococcal exotoxins in skin infiltrating CD4+CD45RO+CD45RA[−] memory T cells and can potentially amplify chronic skin inflammation in AD in the context of bacterial colonization and infection. This should be investigated further in detail in lesional skin of AD and psoriasis patients.

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Introduction

Some years ago a distinct subset of IL-22-producing CD4⁺ T cells, namely T helper (Th)22 cells, were characterized as IL-22-producing T cells different from Th1, Th2 and Th17 cells. These cells concomitantly express the chemokine receptor CCR6 and the skin-homing receptors CCR4 and CCR10 and produce high levels of IL-22, but do not express IL-17 or IFN- γ . Additionally, Th22 cells were found to produce high levels of IL-13 and TNF- α [1–3].

We demonstrated in a previous study that the staphylococcal exotoxins, staphylococcal exotoxin B (SEB) and α -toxin, were strong inducers of IL-22 in CD4⁺ T cells. CD4⁺ T cells from atopic dermatitis (AD) patients secreted significantly more IL-22 compared to psoriasis patients and healthy controls [4]. The aim of this study was to fur-

Table 1. Characterization of human memory T cells and Th22 cells

	IFN- γ , pg/ml	IL-13, pg/ml	IL-17, pg/ml	IL-22, pg/ml
a After 3 days				
Memory T cells	48.16 $\pm$ 18.9 (n = 13)	463.36 $\pm$ 171.0 (n = 14)	0.0 $\pm$ 0 (n = 13)	2.0 $\pm$ 1.5 (n = 16)
Th22 cells generated by long-term cell culture with IL-2 and anti-CD3/anti-CD28	48.45 $\pm$ 9.2 (n = 29)	1,374.77 $\pm$ 186.4 (n = 29)	51.2 $\pm$ 13.8 (n = 28)	588.6 $\pm$ 128.8 (n = 31)
Th22 cells generated with TNF- α and IL-6	50.77 $\pm$ 8.8 (n = 29)	1,558.5 $\pm$ 254.1 (n = 28)	70.8 $\pm$ 22.1 (n = 29)	719.9 $\pm$ 166.2 (n = 32)
b After 6 days				
Memory T cells	47.7 $\pm$ 17.1 (n = 13)	415.5 $\pm$ 190.8 (n = 12)	0.0 $\pm$ 0 (n = 14)	225.4 $\pm$ 219.0 (n = 17)
Th22 cells generated by long-term cell culture with IL-2 and anti-CD3/anti-CD28	46.3 $\pm$ 6.6 (n = 27)	2,612.0 $\pm$ 425.5 (n = 26)	81.9 $\pm$ 26.1 (n = 26)	908.9 $\pm$ 355.0 (n = 28)
Th22 cells generated with TNF- α and IL-6	47.1 $\pm$ 7.4 (n = 29)	3,107.8 $\pm$ 660.5 (n = 27)	103.3 $\pm$ 22.2 (n = 29)	750.0 $\pm$ 154.6 (n = 31)

Data are shown as mean values $\pm$ SEM after 3 or 6 days of cell culture. Th1 (IFN- γ), Th2 (IL-13), Th17 (IL-17) and Th22 (IL-22) cytokine secretion in human CD4+ memory T cells, Th22 cells generated by long-term cell culture with IL-2 and anti-CD3/anti-CD28 and Th22 cells generated with TNF- α and IL-6. Cell-free culture supernatants were quantified for IFN- γ , IL-13, IL-17, and IL-22 secretion by ELISA.

ther investigate IL-22 secretion upon stimulation with staphylococcal exotoxins in human peripheral blood-derived CD4+CD45RO+CD45RA– memory T cells and polarized Th22 cells since highly activated memory T cells are enriched in inflamed AD skin, favor a tissue phenotype and are distinct from freshly isolated CD4+ T cells.

Material and Methods

Patients

Peripheral blood samples were taken from healthy donors and patients with exacerbated psoriasis (n = 9) and AD (n = 8), who presented to our outpatient and inpatient department as previously described [5]. AD was determined by the diagnostic criteria of Hanifin and Rajka [6]. The study was approved by the local ethics committee (No. 5627) and was in accordance with the protocols of the Declaration of Helsinki. All subjects gave informed consent.

Further information about patients and methods is given in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000367923).

Results

Characterization of Human Memory T Cells and Th22 Cells

Th22 cells from CD4+CD45RO+CD45RA– memory T cells cultivated in the presence of IL-6 and TNF- α secreted more IL-22 compared to Th22 cells generated by long-term cell culture with IL-2 and anti-CD3/anti-CD28 or freshly isolated peripheral blood memory T cells after 3 days of cell culture as assessed by ELISA (table 1a). After 6 days of cell culture Th22 cells generated with IL-2 and

anti-CD3/anti-CD28 had an enhanced capacity to produce IL-22 compared to Th22 cells generated in an IL-6- and TNF- α -dependent manner and freshly isolated memory T cells (table 1b). Th22 cells coexpressed IL-13 and are therefore considered as Th22/Th2 cells (table 1).

Intracellular flow cytometric analysis revealed an increase in IL-22 production in Th22 cells generated by long-term cell culture with IL-2 and anti-CD3/anti-CD28 (5.5%) and in Th22 cells cultivated in the presence of IL-6 and TNF- α (5.7%) compared to freshly isolated memory T cells (0.2%; online suppl. fig. S1).

Induction of IL-22 by SEB and α -Toxin in Human CD4+ Memory and Th22 Cells at the mRNA Level

IL-22 expression after SEB and α -toxin stimulation was investigated at the mRNA level by quantitative RT-PCR.

CD4+CD45RO+CD45RA– memory T cells and Th22 cells were either left unstimulated or stimulated with SEB or α -toxin, respectively, for 4 and 8 h. Stimulation with α -toxin or SEB yielded a significant upregulation of IL-22 mRNA in freshly isolated peripheral blood memory T cells (fig. 1a), as well as in Th22 cells derived from memory T cells in long-term cell culture without Th22-promoting conditions (fig. 1b) and Th22 cells cultured with TNF- α plus IL-6 (fig. 1c).

SEB induced a mean 7,095-fold increase in IL-22 mRNA expression in freshly isolated peripheral blood memory T cells (fig. 1a), whereas a median 356- and 334-fold increase in IL-22 mRNA expression was induced after 4 h of SEB stimulation in Th22 cells derived from memory T cells in long-term cell culture without polarization (fig. 1b) and Th22 cells generated with IL-6 and TNF- α

Fig. 1. IL-22 is induced after 4 and 8 h of SEB (100 ng/ml) and α -toxin (50 ng/ml) stimulation in freshly isolated peripheral blood memory T cells (**a**; n = 4–5) as well as in Th22 cells derived from memory T cells in long-term cell culture without polarization

(**b**; n = 20–21) and Th22 cells polarized with IL-6 and TNF- α (**c**; n = 21) at the mRNA level (normalized ratio). Data are shown as mean IL-22/GAPDH ratio + SEM. * p < 0.05; *** p < 0.001.

(fig. 1c), respectively, compared to the medium control. α -Toxin stimulation of 4 h yielded a median 433-fold increase in IL-22 mRNA expression in freshly isolated peripheral blood memory T cells (fig. 1a), whereas IL-22 mRNA was increased approximately 50-fold in Th22 cells derived from memory T cells in long-term cell culture without Th22 promotion (fig. 1b) and 41-fold in Th22 cells generated with IL-6 and TNF- α (fig. 1c), respectively, upon stimulation with α -toxin compared to the appropriate medium control. Therefore, IL-22 expression could neither be enhanced in Th22 cells derived from memory T cells by long-term cell culture without polarization nor in Th22 cells generated with IL-6 and TNF- α upon stimulation with staphylococcal exotoxins compared to freshly isolated peripheral blood memory T cells.

No differences in IL-22 induction upon stimulation with staphylococcal exotoxins were observed between cells from AD patients and cells from psoriasis patients and healthy controls (data not shown).

IL-22 Secretion Is Upregulated in Human CD4+CD45RO+CD45RA– Memory and Th22 Cells after Stimulation with SEB and α -Toxin

To further evaluate IL-22 secretion after SEB and α -toxin stimulation at the protein level, we performed ELISA.

CD4+ memory and polarized Th22 T cells were either left unstimulated or were stimulated with SEB or α -toxin for 72 and 144 h. Freshly isolated peripheral blood memory T cells (fig. 2a), Th22 cells derived from memory T cells in long-term cell culture without Th22-promoting conditions (fig. 2b) and Th22 cells generated with IL-6 and TNF- α (fig. 2c) secreted significantly more IL-22

upon 72 and 144 h of SEB and α -toxin stimulation than the appropriate medium control. SEB and α -toxin stimulation for 144 h did not enhance IL-22 secretion compared to stimulation of 72 h. SEB and α -toxin had the highest capacity of IL-22 induction in freshly isolated peripheral blood memory T cells (fig. 2a) compared to Th22 cells derived from memory T cells in long-term cell culture without Th22-promoting conditions (fig. 2b) and Th22 cells generated with IL-6 and TNF- α (fig. 2c). No differences in IL-22 secretion upon stimulation with staphylococcal exotoxins were observed between cells from AD patients and cells from psoriasis patients and healthy controls (data not shown).

Discussion

In a previous study we demonstrated that SEB and sublytic concentrations of α -toxin are strong inducers of IL-22 in peripheral blood mononuclear cells (PBMCs), T cells and autologous cocultures of keratinocytes and CD4+ T cells. Moreover, we showed an enhanced IL-22 secretion by PBMCs and CD4+ T cells obtained from AD patients compared to psoriasis patients and healthy controls upon stimulation with α -toxin. This observation was significant in T cells from AD patients compared to psoriasis patients and healthy controls [4]. As evidence has increased that Th22 cells play an important role in chronic inflammatory skin disorders, namely AD and psoriasis, we investigated in the present study whether the staphylococcal exotoxins SEB and α -toxin were sufficient to stimulate IL-22 secretion in polarized peripheral blood-derived memory Th22

Fig. 2. Increased IL-22 secretion in freshly isolated peripheral blood memory T cells (**a**; $n = 14$ left, $n = 13$ right) in Th22 cells derived from memory T cells in long-term cell culture (**b**; $n = 21$ – 24 left, $n = 18$ right) and Th22 cells generated with IL-6 and TNF- α (**c**; $n = 22$ – 23 left, $n = 18$ – 20 right) induced by staphylo-

coccal exotoxins (SEB and α -toxin). Cells were either left unstimulated (medium control) or were stimulated for 72 and 144 h with α -toxin or SEB as indicated. Cell-free culture supernatants were quantified for IL-22 secretion by ELISA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

cells since highly activated memory T cells are enriched in inflamed AD skin [7], favor a tissue phenotype and are distinct from freshly isolated CD4 $^{+}$ T cells.

Duhen et al. [2] identified Th22 cells as a distinct subset of CD4 $^{+}$ effector T cells. Moreover, they showed that 5 days

of cell culture of human naïve CD4 $^{+}$ T cells with anti-CD3/anti-CD28 in the presence of IL-6 and TNF- α boosted the differentiation of Th22 cells and concluded that IL-6 and TNF- α enhance Th22 differentiation. Beside IL-22, IL-17 and IFN- γ were investigated as putative coproducing cyto-

kines [2, 8]. We could show that 3 days of cell culture of human memory T cells with IL-2 and anti-CD3/anti-CD28 could induce more IL-22 secretion compared to freshly isolated memory T cells and that adding IL-6 and TNF- α to the cell culture could further enhance this effect, as previously shown by Duhen et al. [2] for naïve T cells. We could further reveal that 6 days of cell culture enhanced IL-22 secretion in human memory T cells with and without IL-2 and anti-CD3/anti-CD28 as well as in T cells stimulated with IL-6 and TNF- α . However, in contrast to Duhen et al., Th22-promoting conditions with IL-6 and TNF- α failed to further enhance IL-22 secretion in human memory T cells after 6 days of cell culture compared to memory T cells generated with IL-2 and anti-CD3/anti-CD28 (table 1b). It is tempting to hypothesize whether this is due to T cell overstimulation with consecutive exhaustion. We could confirm that under Th22-promoting conditions with IL-6 and TNF- α , memory Th22 cells secreted only low amounts of IFN- γ and IL-17 [2]. However, memory Th22 cells coproduced high amounts of IL-13 in our hands and are therefore considered as Th22/Th2 cells, whereas this cytokine was not investigated by Duhen et al. in naïve T cells. Trifari et al. [1] described a population of CCR4+CCR6+CCR10+ human memory T cells that produced IL-22 and IL-13 but not IFN- γ or IL-17 and called them Th22 cells, which is in line with our data.

Here we could show that SEB and α -toxin strongly induced IL-22 secretion in freshly isolated peripheral blood memory T cells as well as in Th22 cells derived from memory T cells in long-term cell culture without polarization and Th22 cells generated under Th22-promoting conditions with IL-6 and TNF- α . Although IL-22 secretion was higher in Th22 cells compared to freshly isolated memory T cells (online suppl. fig. S1), IL-22 induction by staphylococcal exotoxins did not differ between these three cell types (fig. 2a–c). This implicates

the following: (1) in vivo existing memory T cells are able to produce similar amounts of IL-22 upon staphylococcal exotoxin stimulation compared to in vitro highly polarized Th22 cells and (2) skin-infiltrating memory T cells can produce IL-22 rapidly upon contact with staphylococcal exotoxins.

Interestingly, α -toxin stimulation increased IL-22 secretion of PBMCs and CD4+ T cells obtained from AD and psoriasis patients and healthy controls in our previously published work [4]. We could not detect any differences in staphylococcal exotoxin-induced IL-22 secretion between blood-derived Th22 cells from AD patients and cells from psoriasis patients and healthy controls in the present study. It is tempting to hypothesize whether long-term T cell culture that is necessary for Th22 polarization can mask inflammatory in vivo conditions and whether it is rather artificial than suitable for investigating chronic inflammatory skin diseases such as AD and psoriasis. The role of Th22 cells, including related cytokines and transcription factors, in the context of staphylococcal colonization and infection in AD should be further investigated directly in the skin compartment to elucidate its role in the pathogenesis and maintenance of eczema.

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Disclosure Statement

The authors have no financial conflicts of interest to declare.

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