The impaired suppressive activity and altered response to IL-33 of regulatory T cells in a psoriasis model may be due to decreased ST2 expression

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Introduction

Interleukin 33 (IL-33) is a unique cytokine with regard to its structure, localization and function. Initially described as an alarmin, released by cells following cell damage, IL-33 has been shown to fulfill numerous functions in various infections and inflammatory diseases by modulating both the innate and the adaptive immune system. But IL-33 can also act in an immunosuppressive fashion by inducing regulatory T cells (Treg). We observed that disruption of the skin barrier by tape stripping in mice induced IL-33. In addition, the contact hypersensitivity (CHS) was found suppressed which was due to the induction of Treg by IL-33. Accordingly, blockade of IL-33 enhanced CHS, whereas injection of IL-33 suppressed CHS via induction of Treg. Enhanced IL-33 levels can be found both in the skin and the serum of psoriatic patients. On the other hand, in psoriasis Treg appear to be impaired in their suppressive function. Thus, we asked whether “psoriatic” Treg are impaired in their response to IL-33 in the imiquimod (IMQ)-induced psoriasis like model. IL-33 was enhanced in skin and serum of IMQ-mice. To study the influence of IL-33 on Treg in IMQ-mice, CD4⁺CD25⁺ Treg isolated from IMQ or untreated mice were injected into naïve mice which were sensitized 24 h later. Ear swelling was reduced in mice receiving Treg from untreated donors, whereas injection of Treg from IMQ-mice did not cause suppression, implying impairment of Treg in IMQ-mice. To study the response of Treg to IL-33 in IMQ-mice, Treg from untreated or IMQ-mice were incubated with IL-33 and injected into naïve mice which were sensitized thereafter. The suppressive capacity of Treg from WT mice was further enhanced by IL-33, whereas Treg from IMQ mice did not to respond to IL-33. Furthermore, the expression of IL-33 binding receptor (ST2) was downregulated on Treg obtained from IMQ-treated mice. The mechanisms by which ST2 expression is down-regulated in the IMQ-model (and-or psoriasis) remain to be elucidated.

Results

IL-33 expression is elevated in both skin and serum of IMQ-treated mice

To prove whether IL-33 expression, analogous to psoriasis patients, is elevated in mice treated with IMQ for 5 days, immunofluorescence staining of IL-33 and ELISA were performed and revealed that IL-33 expression was elevated in both skin and serum from C57BL/6J wildtype treated with IMQ (Fig.1a). Furthermore, the expression of the key cytokines that determine psoriasis in humans, IL-6 and IL-17, was strongly upregulated as determined by quantitative real-time PCR (qRT-PCR) of RNA obtained from IMQ-treated and naïve skin (Fig.1b).

Fig. 1. (a) Immunofluorescence staining of IL-33 in naïve and IMQ-treated mice compared to IgG control. IL-33 ELISA from serum of IMQ-treated and naïve mice. *p Student <0.05. (b) Quantitative real-time PCR for the transcription analysis of IL-6 and IL-17 from RNA obtained from the skin of IMQ-treated and naïve mice. *p Student <0.05.

Activity of Treg obtained from IMQ-treated mice is not enhanced by IL-33

To study whether the activity of Treg is influenced by the enhanced levels of IL-33 in IMQ-mice, CD4⁺CD25⁺ Treg were obtained from IMQ or untreated mice. Cells were injected into naïve mice which were sensitized 24 h thereafter. Ear challenge was significantly reduced in mice receiving Treg from untreated mice in comparison to positive control mice which were sensitized and challenged only. In contrast, no suppression was observed upon injection of Treg obtained from IMQ-mice, suggesting that in IMQ-mice Treg are impaired in their function (Fig. 2a). To study whether the response of Treg to IL-33 is altered in IMQ-mice, Treg from untreated or IMQ-mice were incubated with IL-33 for 24 h and injected into naïve mice which were sensitized thereafter. The suppressive capacity of Treg obtained from WT mice could be further enhanced by IL-33, whereas Treg from IMQ mice do not respond to IL-33. (Fig. 2b).

Fig.2 (a) CD4⁺CD25⁺ Treg were isolated from spleens and lymph nodes from naïve sensitized and/or IMQ-treated mice and injected into naïve WT mice (8x10⁵ cells), which were sensitized with TNCB 24 hours later. Five days thereafter, ears were challenged with the same hapten and ear swelling was measured 24 hours later. CHS was determined as the thickness of the hapten-challenged ear compared with the thickness of the vehicle-treated ear and expressed in cm × 10⁻³ (mean ± standard deviation). pANOVA <0.001; *pStudent <0.05 vs PosCo; ns = not significant. (b) Treg were isolated from sensitized and/or naïve mice and IMQ-treated animals and cultured with 10 ng/ml recombinant IL-33 for 24 hours or left untreated. Cells (8x10⁵) were injected intravenously into naïve recipients which were sensitized with TNCB 24 hours later. Five days thereafter, ears were challenged with the same hapten and ear swelling was measured 24 hours later. CHS was determined as the thickness of the hapten-challenged ear compared with the thickness of the vehicle-treated ear and expressed in cm × 10⁻³ (mean ± standard deviation). p ANOVA <0.001; *p Student <0.05 vs PosCo; **p Student <0.05 vs Treg Co w/o.

ST2 expression on IMQ-Treg is downregulated

IL-33 exerts its activity via binding to its receptor ST2. ST2 expression identifies a suppressive activated Treg. FACS analysis of Foxp3⁺CD4⁺CD25⁺ cells revealed that ST2 expression on IMQ-treated mice is downregulated in comparison to Treg cells obtained from untreated animals (Fig.3.).

Fig.3. CD4⁺CD25⁺ Treg cells were isolated from lymph nodes and spleens of IMQ-treated or naïve animals using CD4⁺CD25⁺Regulatory T Cell Isolation Kit (Miltenyi Biotec). Two color staining was conducted using an APC-coupled Foxp3 monoclonal antibody (Invitrogen) and biotin anti mouse ST2 antibody. As a second step Alexa Fluor-488 streptavidin was used. (both from BioLegend).

Summary

- Expression of IL-33 is enhanced in the IMQ-induced psoriasis-like mouse model.
- The proinflammatory cytokines IL-6 and IL-17 are significantly upregulated in the skin of IMQ-treated mice.
- Despite the enhanced expression of IL-33, Treg activity is reduced in the IMQ-model in contrast to the CHS-model.
- Decreased expression of the IL-33 receptor ST2 could be an explanation for the weaker response of Treg to IL-33 in IMQ-treated mice

Conclusion

T cells from IMQ-treated mice are impaired in their function. This may be associated with an altered response to IL-33. Future studies have to elucidate the mechanisms by which ST2 expression is down-regulated in the IMQ-model and whether reduced ST2 expression can be also observed in psoriasis.