

Contact allergens induce CD8⁺ T cell-derived interleukin 10 that appears dispensable for regulation of contact hypersensitivity

Abstract

Interleukin 10 (IL-10) has been implied in the regulation of allergic contact dermatitis. Using transcriptional reporter mice we analyzed cellular sources of IL-10 during contact hypersensitivity (CHS) and identified IL-10 expressing CD8⁺ T cells in the skin that are antigen-specific, display PD-1, an effector memory phenotype, and IL-10 expression comparable to that of CD4⁺ T cells. However, in mice with a selective IL-10 deficiency in CD8⁺ T cells CHS responses were comparable to that of controls, even in the absence of CD4⁺ cells, suggesting that CD8⁺ T cell-derived IL-10 does not contribute significantly to the resolution of CHS responses.

1 | BACKGROUND

Allergic contact dermatitis (ACD) is a common skin disease caused by skin contact with chemicals that induce T-cell-mediated inflammation and cytotoxicity.^[1,2] While the processes of sensitization and elicitation are well investigated using the mouse model of contact hypersensitivity (CHS), mechanisms that determine the resolution of the inflammatory response are less well understood. Previously, it has been suggested that IL-10 plays a crucial role in the resolution of ACD; however, the exact source of IL-10 in the skin remained elusive^[3] (s1, s2).

2 | QUESTIONS ADDRESSED

To investigate in more detail the cellular sources of IL-10 during CHS in skin and draining lymph nodes.

3 | EXPERIMENTAL DESIGN

CHS to DNFB was studied in IL-10 transcriptional reporter (Vert-X) mice^[4] and mice with selective IL-10 deficiency in CD8⁺ T cells (IL-10^{FL/FL} E8₁-Cre⁺ mice).^[5,6]

For full details of materials and methods, see Data S1.

4 | RESULTS

Compared to wild-type mice, Vert-X mice had a normal CHS response to DNFB (Fig. S1). More than 85% of the IL-10-expressing cells in the skin were T cells (Figure 1A). Analysis of the IL-10-eGFP expression in T cells isolated from ear skin at 24, 72 and 120 h after challenge revealed a CD8⁺ IL-10-expressing T cell population in addition to the expected IL-10-expressing CD4⁺ T cells (Figure 1B,C). This was not evident in skin-draining lymph nodes where IL-10 expression was predominantly detected in CD4⁺ T cells (Fig. S2a,b). IL-10-eGFP⁺ CD8⁺ T cells in the skin had an effector memory phenotype^[7] and characteristics of Treg cells^[8] (Fig. S3a,b). They were most prominent during the resolution phase of CHS. IL-10 expression peaked at 72 h with similar levels as in CD4⁺ T cells (Figure 1D). We also detected IL-10-expressing CD8⁺ T cells in CHS to other contact allergens such as TNCB and oxazolone (Fig. S4). The skin-derived IL-10-eGFP CD8⁺ T cells secreted IL-10 (Figure 1E) and proliferated in an antigen-specific manner (Figure 1F). Finally, depletion of CD4⁺ T cells prior to hapten challenge demonstrated that the IL-10-eGFP expression of CD8⁺ T cells was independent of CD4⁺ T-cell help (Fig. S5).

Substantial IL-10 expression of CD8⁺ T cells suggested that they may contribute to the regulation of the CHS response. However, the CHS response in mice that displayed selective IL-10 deficiency in CD8⁺ T cells (Fig. S6) (IL-10^{FL/FL} E8₁-Cre⁺ mice)^[5,6] was comparable to that of Cre⁻ littermates when challenged with optimal (Figure 2A, left)

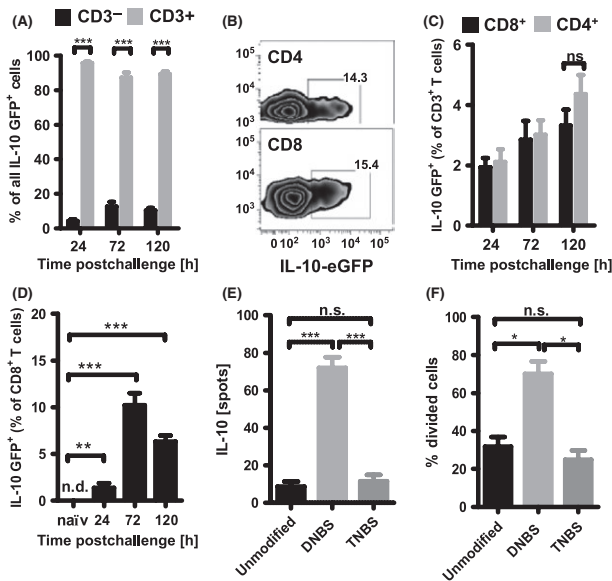


FIGURE 1 CHS response to DNFB induces IL-10-eGFP expression in CD4⁺ and CD8⁺ T cells in the skin. Vert-X mice were sensitized and challenged with DNFB, and skin cells were analysed for IL-10-eGFP expression at 24, 72 and 120 h after challenge via flow cytometry. (A) Relative distribution of IL-10-eGFP expression in CD3⁺ and CD3⁻ cells; (B) representative contour plot of IL-10-eGFP expression CD8⁺ and CD4⁺ cells of ear skin 72 h after hapten application; (C) percentage of IL-10-eGFP⁺ CD4⁺ and CD8⁺ cells among the CD3⁺ T-cell population; (D) percentage of IL-10-eGFP⁺ cells among CD8⁺ T cells; (E) IL-10 secretion of CD8⁺ T cells isolated from s.dLN of DNFB-sensitized and DNFB-challenged mice after 72 h of re-stimulation with unmodified, DNBS-modified or TNBS-modified BMDCs as analysed via ELISPOT assay (mean±SEM of two experiments, n=8); (F) antigen-specific proliferation of IL-10-eGFP⁺ CD8⁺ T cells from DNFB-sensitized Vert-X mice after 72 h of re-stimulation with unmodified, antigen-specific modified (DNBS) or antigen-unspecific modified BMDCs (TNBS) (mean±SEM of two experiments, n=5)

and suboptimal (Figure 2A, right) hapten concentrations. This was also observed following antibody-mediated depletion of CD4⁺ T cells prior to challenge (Figure 2B).

5 | CONCLUSIONS

Our findings identify CD8⁺ T cells as an additional source of IL-10 in the resolution phase of CHS. These CD8⁺ T cells may derive from the effector T-cell pool as some of them co-express IFN- γ and IL-10. They exhibit an effector memory phenotype (CD44^{hi}CD62L⁻) as well as features of regulatory T cells (Treg) (ICOS+PD-1⁺). The expression of IL-10 in CD8⁺ T cells that co-express ICOS, PD-1 and in part IFN- γ suggests that these may act in concert with conventional Tregs in immune regulation, similar to findings reported for viral or bacterial infections^[9] (s3, s4). However, at least under the conditions tested, selective IL-10 deficiency in CD8⁺ T cells did not result in an enhanced or prolonged ear swelling response, suggesting that CD8⁺ T-cell-derived IL-10 is dispensable for the regulation of contact hypersensitivity.

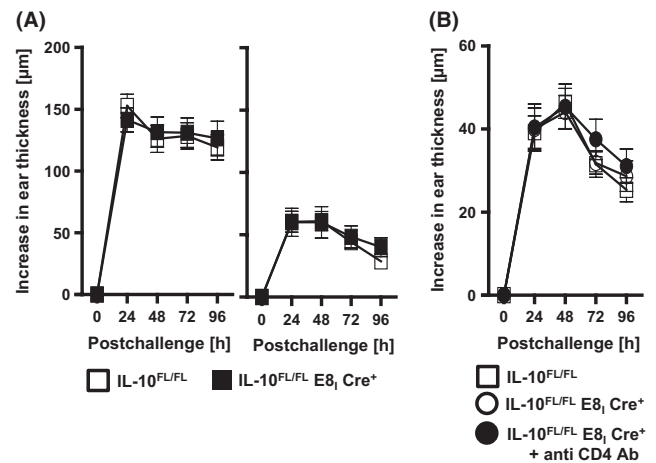


FIGURE 2 Mice with IL-10 deficiency of CD8⁺ T cells develop a normal CHS response. (A) IL-10^{FL/FL} E8_i-Cre⁺ and IL-10^{FL/FL} mice were sensitized and challenged with DNFB 0.2% (left) (mean±SEM of three experiments, n=24) or with a suboptimal concentration of 0.05% (right) (mean±SEM of two experiments, n=18). (B) CHS reaction after challenge with suboptimal DNFB concentration in IL-10^{FL/FL} mice and in IL-10^{FL/FL} E8_i-Cre⁺ mice with or without CD4 T cell depletion (anti-GK1.5 antibody) prior to challenge (mean±SEM of two experiments, n=11-14)

ACKNOWLEDGEMENTS

The technical assistance of Ursula Voith and Eva Bachtanian, Allergy Research Group, Freiburg, is greatly acknowledged. We thank Christopher L. Karp (Division of Molecular Immunology, Cincinnati Children's Hospital Research Foundation, Cincinnati) for providing IL-10 transcriptional reporter mice and Ichiro Taniuchi (RIKEN Research Center for Allergy and Immunology, Yokohama, Japan) for providing access to E8_i-Cre mice and Andreas Thiel (Berlin-Brandenburg Center for Regenerative Therapies, Charité University Medicine, Berlin, Germany) for providing breeding pairs.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

AD, SK and BD performed the experimental work. AR provided the IL-10^{FL/FL} mice and discussed the data. TJ initiated and designed the study, and SFM and TJ supervised the project and wrote the manuscript. All authors contributed to revising the manuscript and approved the final version.

Keywords

allergic contact dermatitis, contact hypersensitivity, immune regulation, skin, T cell

Britta Dorn^{1,3}
 Axel Roers⁴
 Stefan F. Martin¹
 Thilo Jakob^{1,3}

¹Allergy Research Group, Department of Dermatology, Medical Center—
 University of Freiburg, Freiburg, Germany

²Faculty of Biology, University of Freiburg, Freiburg, Germany

³Department of Dermatology and Allergology, Justus Liebig University
 Giessen, University Medical Center Giessen (UKGM), Giessen, Germany

⁴Institute of Immunology, Medical Faculty Carl Gustav Carus, Technische
 Universität Dresden, Dresden, Germany

Correspondence

Thilo Jakob, Department of Dermatology and Allergology, University
 Medical Center Giessen (UKGM), Justus-Liebig-University, Giessen,
 Germany.

Email: thilo.jakob@derma.med.uni-giessen.de

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

- FIGURE S1** Vert-X mice display a normal CHS response to DNFB
- FIGURE S2** DNFB induces IL-10-eGFP expression in skin draining lymph node cells
- FIGURE S3** IL-10-eGFP⁺CD8⁺ T cells of the skin show an activated, effector memory phenotype
- FIGURE S4** CHS response to TNCB or oxazolone induces IL-10-eGFP expression in CD8⁺ T cells in the skin
- FIGURE S5** IL-10-eGFP expression of CD8⁺ T cells is independent of CD4⁺ T cell help
- FIGURE S6** IL-10 deficiency in IL-10^{FL/FL} E8₁-Cre⁺ mice is selective for CD8⁺ T cells
- DATA S1** Supplementary methods