OncoImmunology 2:3, e23186; March 2013; © 2013 Landes Bioscience

IL-10 control of dendritic cells in the skin

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Keywords: conditional gene targeting, contact hypersensitivity, dendritic cells, immune regulation, interleukin-10, IL-10 receptor signaling, Langerhans cells, skin

Abbreviations: CHS, contact hypersensitivity; DC, dendritic cell; IL-10, interleukin-10; LC, Langerhans cell; LN, lymph node; sdLN, skin-draining LN; Treg, regulatory T cell; WT, wild type

Interleukin-10 (IL-10) is a potent immunomodulatory cytokine, whose cellular targets have not yet been precisely identified. Dendritic cell (DC)-specific IL-10 receptor knockout mice exhibit exaggerated T-cell reactivation in the skin, highlighting a key role of DCs in the maintenance of local immune homeostasis, beyond their classical function as regulators of T-cell priming in lymph nodes.

In addition to the generation of protective immune responses, effective immune defense strategies depend on intricate negative regulation to restrict tissue damage due to inflammation. Interleukin-10 (IL-10) is an immunosuppressive cytokine produced by and acting on many different leukocytes, including dendritic cells (DCs) and T cells.[1,2] In particular, regulatory T cells (Tregs) exert their immunosuppressive function largely via the secretion of IL-10. As a gatekeeper of excessive immune responses against foreign antigens, IL-10 is expressed at epithelial interfaces to the environment, including the skin. Consequently, *IL10*$^{-/-}$ mice develop increased contact hypersensitivity (CHS),[3] a relevant mouse model for allergic contact dermatitis.[4] Following the topical application of a contact sensitizer (hapten), DCs prime hapten-specific T cells in skin-draining lymph nodes (sdLNs). The subsequent painting of the same hapten onto the ears triggers a transient ear-swelling reaction, driven by interferon γ (IFNγ)-secreting T_H1 cells and regulated by CD4$^+$ T cells producing IL-10. Mice bearing a T cell- or Treg-specific deletion of *IL10* exhibit aggravated CHS, demonstrating the essential role of T cell-derived IL-10 to curtail the inflammatory skin reaction.[5,6] The cellular targets of IL-10 during CHS remain elusive.

DCs have the unique capacity to balance immunity and tolerance and hence are critical in the maintenance of immune homeostasis during infection/inflammation as well as in steady-state conditions.[7] DCs continuously probe their environment and induce robust, protective T-cell responses to pathogen-derived antigens as well as T-cell tolerance to innocuous foreign and self-antigens, for instance via the induction of Tregs. Besides Langerhans cells (LCs) in the epidermis, the skin harbors distinct dermal DC populations, and we recently established the functional redundancy of distinct cutaneous DC subsets in mediating CHS.[8] During hapten sensitization, the production of IL-10 by LCs—and presumably also by dermal DCs—is required to limit ear swelling, as mice bearing a LC-specific *IL10* knockout mount aggravated CHS responses.[9] Conversely, although IL-10 can inhibit DC maturation and skew DCs toward a tolerogenic phenotype in vitro, it is still unknown to what extent IL-10 controls DC function in vivo.

To address this question, we analyzed mice bearing a DC-specific deletion of the gene coding for the IL-10 receptor (*DC-IL10R*$^{-/-}$ mice), both in steady-state conditions and during CHS.[10] We made several observations that shed new light on the molecular control of DCs by IL-10. First, IL-10 signaling in DCs is dispensable to maintain their immature phenotype in steady-state conditions.[10] The surface expression of MHC Class II, co-stimulatory and co-inhibitory molecules was not altered in IL-10R-deficient DCs. Accordingly, T cells in DC-IL10R$^{-/-}$ animals were non-activated and the frequency of Tregs was similar to that of wild type (WT) mice. Second, the control of DC by IL-10 is necessary to prevent aggravated CHS.[10] In contrast to total and Treg-specific *IL10*$^{-/-}$ mice,[3,5,6] *DC-IL10R*$^{-/-}$ and WT mice developed a similar ear-swelling reaction 24 h after hapten challenge. Intriguingly, at 48 h, ear swelling further increased in *DC-IL10R*$^{-/-}$ mice, while the inflammation began to resolve in WT animals. Third, IL-10 controls DC function during the effector phase of CHS.[10] The adoptive transfer of T cells primed in WT or *DC-IL10R*$^{-/-}$ donor mice into either type of recipient animals, confirmed similar ear swelling 24 h after hapten challenge. In contrast, at 48 h the ear-swelling reaction was significantly stronger in DC-IL10R$^{-/-}$ recipients, whereas T cells that were primed by IL-10R-deficient and reactivated by WT DCs failed to elicit

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Submitted: 12/05/12; Accepted: 12/08/12
Citation: Clausen BE, Girard-Madoux MJH. IL-10 control of dendritic cells in the skin. OncoImmunology 2013; 2:e23186; http://dx.doi.org/10.4161/onci.23186

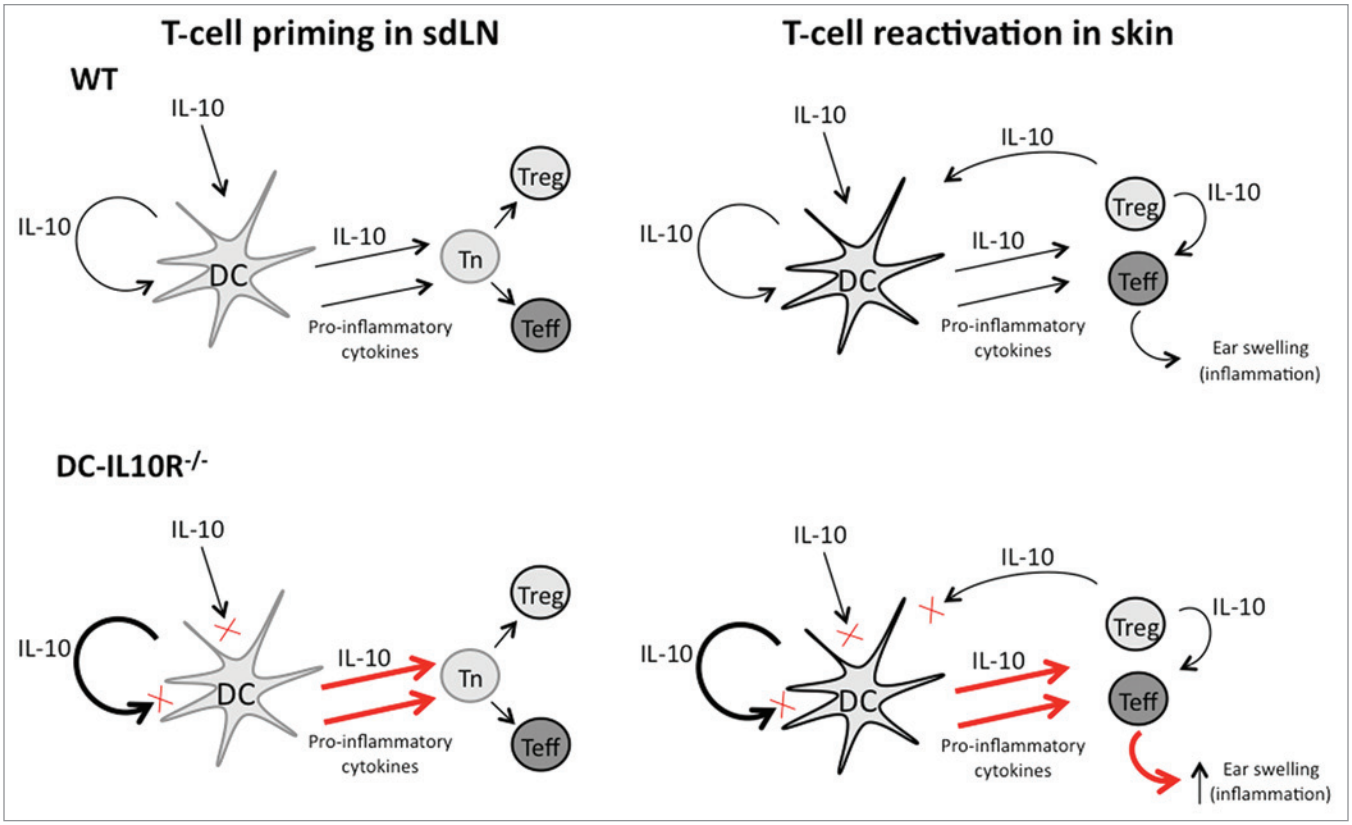

Figure 1. The control of dendritic cells by interleukin-10 is dispensable during naïve T-cell priming in skin-draining lymph nodes, but essential to curtail excessive effector T-cell reactivation in the skin. During the sensitization phase, the topical application of a contact sensitizer (hapten) onto the skin induces the migration of dendritic cells (DCs) into skin-draining lymph nodes (sdLNs) for the priming of naïve hapten-specific T cells (left). The balanced production of interleukin-10 (IL-10) and pro-inflammatory cytokines induces the differentiation of appropriate effector T cells (Teffs) and regulatory T cells (Tregs), despite elevated cytokine secretion by IL-10 receptor-deficient DCs. IL-10 signaling in DCs is not required at the time of T-cell priming. During the elicitation of contact hypersensitivity (CHS), antigen-specific Teffs and Tregs are re-activated by DCs in the skin (right). In the absence of IL-10 signaling in DCs this leads to enhanced ear swelling/inflammation, irrespective of an increased expression of IL-10 in situ. Tn, naïve T cell.

exaggerated ear swelling. Forth, IL-10R-deficient DCs produce elevated amounts of cytokines after in vitro stimulation.[10] Although the activation of *IL10R*$^{-/-}$ and WT DCs by lipopolysaccharide (LPS) induced similar phenotypic maturation, according to surface marker expression, *Il10r*$^{-/-}$ DCs secreted increased amounts of tumor necrosis factor α (TNFα), IL-6 and IL-10. Fifth, enhanced cytokine expression and cellular influx occur in the skin of *DC-IL10R*$^{-/-}$ animals during the elicitation of CHS.[10] As soon as 24 h after a hapten challenge, the expression of pro-inflammatory cytokines and IL-10 was increased in the skin of these animals as compared with their WT counterparts, causing a massive infiltration of innate, MHC Class II^{+} and T cells at 48 h. Whereas the global extent of infiltration was lower in WT mice, the number of skin-infiltrating T cells was similar in both strains. Intriguingly, the increase in T cells observed from 24 to 48 h was mainly caused by the recruitment of FOXP3^{+} Tregs into the skin.

The major implications of these findings are 3-fold (**Fig. 1**). First, the lack of IL-10 signaling in DCs does not alter T-cell priming in sdLNs during hapten sensitization. Most likely, the balanced, albeit elevated secretion of pro-inflammatory cytokines and inhibitory IL-10 by IL-10R-deficient skin-infiltrating DCs drives a similar differentiation of effector cells and Tregs as in WT mice. Therefore, ear swelling in *DC-IL10R*$^{-/-}$ mice is not augmented 24 h after a hapten challenge. Accordingly, the production of IL-10 by LCs, which is essential to prevent exaggerated T-cell priming and ear swelling at 24 h,[9] exclusively acts on naïve T cells, rather than in an autocrine fashion. Moreover, the potential paracrine regulation of mature DC function by IL-10 is dispensable during T-cell priming. Second, DCs are the target of IL-10 to limit excessive T-cell reactivation during the elicitation of CHS, as demonstrated by the exaggerated ear swelling observed 48 h after hapten challenge in *DC-IL10R*$^{-/-}$ mice. In contrast to priming, the IL-10-mediated autocrine/paracrine negative feedback on DCs in situ is required to curtail inflammation and tissue damage. This highlights a critical role of skin DCs in the maintenance of immune homeostasis that goes beyond their classical function as migratory antigen-presenting cells. Whether such DCs are resident and/or inflammatory remains to be determined. Finally, the exaggerated ear swelling observed at 48 h, despite the elevated expression of IL-10 in the skin, indicates that the regulation of T cells by IL-10 alone is inadequate to restrict adaptive immune responses in inflamed

tissues. Therefore, FOXP3$^+$ Tregs preferentially recruited into the skin 48 h after elicitation of CHS exert their regulatory function largely by IL-10 control of DCs and to a lesser extent of effector T cells.

In conclusion, our study establishes that IL-10 signaling in DCs is dispensable during T-cell priming but essential to limit the magnitude and duration of the effector CHS response.[10] This regulatory function of DCs in the skin has significant implications for the immunotherapy of human diseases (e.g., cancer) since the release of DCs from the control mediated by IL-10 might promote their immunogenicity without increasing the risk for autoimmunity due to enhanced T-cell priming.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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