Epidermal IL-15Rα acts as an endogenous antagonist of psoriasiform inflammation in mouse and man

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Stromal cells at epithelial surfaces contribute to innate immunity by sensing environmental danger signals and producing proinflammatory cytokines. However, the role of stromal cells in controlling local inflammation is unknown. We show that endogenous soluble IL-15 receptor α (IL-15Rα) derived from epidermal stroma, notably keratinocytes, protects against dendritic cell/IL-15-mediated, T cell-driven skin inflammation in vivo, and is relevant to human psoriasis. Selective lack of IL-15Rα on stromal epidermal cells exacerbated psoriasiform inflammation in animals. Epidermal IL-15Rα was shed by keratinocytes via proteolytic cleavage by matrix metalloproteinases upon stimulation with proinflammatory cytokines to counteract IL-15-induced proliferation of IL-17+ αβ and γδ T cells and production of TNF, IL-23, IL-17, and IL-22 during skin inflammation. Notably, administration of soluble IL-15Rα was able to repress secretion of IL-1β, IL-6, and TNF by keratinocytes, dampen expansion of IL-17+ αβ and γδ T cells in vivo, and prevent psoriasis in two mouse models, including human xenograft AGR mice. Serum levels of soluble IL-15Rα negatively correlated with disease severity, and levels rose upon successful treatment of psoriasis in patients. Thus, stressed epidermal stromal cells use soluble IL-15Rα to dampen chronic inflammatory skin disease.

Psoriasis is a chronic, relapsing-remitting inflammatory disorder mediated by effector T cells, including CD4+ and CD8+ αβ and γδ T cells (Lowes et al., 2007; Nestle et al., 2009). These T cells secrete an array of proinflammatory cytokines, including tumor necrosis factor-α (TNF), interferon-γ (IFN-γ), IL-17, and IL-22, to stimulate proliferation of keratinocytes and recruit inflammatory cells (Di Meglio et al., 2011; Lowes et al., 2007). Notably, αβ T cells appear to be crucial in the chronic-relapsing phase of the disease, where these cells become resident in the skin, presumably as memory T cells dependent on IL-15 and other homeostatic cytokines (Boymàn et al., 2007). Upon induction of a psoriatic lesion, CD8+ T cells, notably αβ, integrin+ cells, home to the epidermis and correlate with epidermal thickening (acanthosis) and elongation of dermal papillae (papillomatosis), leading to enhanced interdigitation of epidermis and dermis (Boymàn et al., 2004; Conrad et al., 2007). As for γδ T cells, the involvement of these cells has recently been implicated in the pathogenesis of psoriasis (Laggner et al., 2011), especially during the early stages of psoriasiform skin inflammation in mouse models (Cai et al., 2011; Pantelyushin et al., 2012).

Furthermore, DCs appear to be involved very early in the pathogenesis of psoriasis by producing type I IFNs (Nestle et al., 2005). Interestingly, treatment with imiquimod (IMQ), an agonist of Toll-like receptors (TLR) 7 and 8, can stimulate DCs to produce type I IFNs, and thus exacerbate psoriasis in patients (Gilliet et al., 2004). Moreover, treatment of normal mice with IMQ results in psoriasiform skin inflammation, which is characterized by epidermal thickening and interdigitation of epidermis and dermis, thus resembling papillomatosis (van der Fits et al., 2009).
Cutaneous inflammation in the IMQ model appears to share many pathophysiologic pathways with (early) psoriasis plaque formation (Nestle et al., 2009). Hence, the immune cascade involved in this IMQ-induced psoriasis model hinges on the stimulation of DCs via TLR7 and 8, leading to activation of the IL-23–IL-17 axis with stimulation of T cells capable of IL-17 and IL-22 production (van der Fits et al., 2009; Cai et al., 2011; Pantelyushin et al., 2012; Tortola et al., 2012). The importance of IL-17 in psoriasis is further highlighted by the recent success of biologics targeting IL-17 in patients (Krueger, 2012; Leonardi et al., 2012; Papp et al., 2012).

In addition to the immune system, keratinocytes have long been known to produce proinflammatory cytokines, including TNF and IL-1β, thus contributing to the inflammatory milieu in psoriatic skin plaques (Barker et al., 1991). Moreover, a publication examining the expression of IL-15 and IL-15Rα on keratinocytes in vitro, as well as IL-15 and IL-15-binding sites in skin biopsies from psoriasis patients, suggested that keratinocytes might stimulate neighboring keratinocytes and immune cells by presenting IL-15 via membrane-bound IL-15Rα to these cells (Rückert et al., 2000). Recently, production of the proinflammatory IL-1 family member II-36 by keratinocytes has been shown to play a role in inducing psoriasiform inflammation in the IMQ mouse model (Tortola et al., 2012). Furthermore, keratinocytes are known to secrete different antimicrobial peptides, including β-defensins, LL-37 cathelicidin, and S100 proteins. Some of these antimicrobial peptides are able to form complexes with self-DNA molecules, thereby leading to the activation of complexes with DCs, as has been shown for LL-37–DNA complexes stimulating plasmacytoid DCs via TLR9 engagement to produce IFN-α (Lande et al., 2007). Moreover, antimicrobial peptides exert chemotactic activities toward innate and adaptive immune cells. Thus, keratinocytes, through the production of antimicrobial peptides and proinflammatory cytokines, are contributing to cutaneous inflammation in immune defense, as well as in psoriasis (Nestle et al., 2009). However, whether keratinocytes or other stromal cells are involved in modulating or dampening inflammation in the skin is unknown.

Here, we show that stromal cells, most notably keratinocytes in the skin, express high levels of IL-15Rα and release soluble IL-15Rα (sIL-15Rα) via proteolytic cleavage to suppress the inflammatory response in murine and human psoriasis. These data reveal a crucial role for stroma-derived sIL-15Rα in counter-regulating inflammation in this common chronic inflammatory disorder.

**RESULTS**

**Opposing effects of IL-15 and IL-15Rα in psoriasiform skin inflammation**

IL-15 can induce the production of proinflammatory cytokines involved in the pathogenesis of psoriasis (McInnes and Liew, 1998). Hence, it was not surprising that administration of IMQ to II15a−/− mice failed to induce a psoriasiform skin pathology, in contrast to WT mice, which showed marked thickening of epidermis and interdigitation of epidermis and dermis of the treated back or ear skin compared with control WT mice treated with Vaseline (Fig. 1 A). II15−/− mice showed consistently less thickening of the IMQ-treated ears compared with WT, which was already evident on day 4 of IMQ application (Fig. 1 B). On day 6, ear thickness, epidermal thickness, and interdigitation of epidermis and dermis of II15−/− mice was 35–50% lower than in WT animals (Fig. 1, C–E).

These results were in line with a previous study reporting the use of a mAb interfering with the binding of IL-15 to IL-15Rα, thus inhibiting IL-15R signaling and the maintenance of psoriasiform inflammation in a xenograft mouse model (Villadsen et al., 2003). Thus, we expected to see a similar phenotype as in II15−/− mice when treating II15a−/− mice with IMQ. Very surprisingly, however, II15a−/− animals were not only able to develop a psoriasiform disease upon IMQ application, but the inflammatory skin reaction was significantly more pronounced than in WT mice (Fig. 1 A). Ear thickness of IMQ-treated II15a−/− animals was increased as early as on day 3 (Fig. 1 B), and, on day 6 of IMQ treatment, ear thickness, epidermal thickness and interdigitation of epidermis and dermis was 75–130% higher in II15a−/− compared with WT mice (Fig. 1, C–E).

**Psoriasiform skin disease depends on IL-15 production by DCs**

Immunohistochemical analysis of skin sections from WT and II15a−/− animals for IL-15 revealed the presence of IL-15+ cells in the dermis (unpublished data). To gain insight into which cells produced IL-15 upon cutaneous IMQ application, we generated BM chimeras using WT or II15a−/− BM cells adoptively transferred into lethally irradiated WT or II15−/− hosts. Upon de novo reconstitution of the immune compartment and IMQ treatment, these BM chimeras showed that absence of IL-15 on radio-resistant host stromal cells was dispensable for skin inflammation (Fig. 2, A and B). Conversely, selective deficiency of IL-15 on hematopoietic cells as found in II15a+/− hosts was sufficient to replicate the phenotype of II15−/− mice (Fig. 2, A and B).

As IMQ is a TLR7 and 8 agonist, and these TLRs are abundant in DCs, we reasoned that IL-15 production by these cells might be crucial for IMQ-mediated psoriasiform pathology. Thus, selective depletion of DCs in Cd11cDtr mice receiving diphtheria toxin (DT) significantly reduced skin inflammation upon IMQ application (unpublished data), in line with a recent publication (Tortola et al., 2012). To exclude the possibility that CD11c+ DCs produced another intermediary cytokine acting on another cell subset to induce IL-15 production by the latter, we generated chimeras carrying a 1:1 mixture of II15−/− and Cd11cDtr BM adoptively transferred to lethally irradiated WT hosts. In these mice, II15−/− DCs are resistant to DT treatment but unable to produce IL-15, whereas Cd11cDtr are the only DCs able to secrete IL-15. Upon IMQ treatment, mixed chimeras receiving DT showed a similar deficiency to develop psoriasiform inflammation (Fig. 2 C), as did II15−/− mice and DT-treated Cd11cDtr animals. Conversely, mixed II15−/−→Cd11cDtr chimeras receiving PBS developed robust...
This was not caused by the persistence of radio-resistant skin DCs expressing lower CD11c levels, including Langerhans cells, the latter of which have been shown to resist high-dose irradiation (Merad et al., 2002). Thus, upon IMQ treatment, \( \text{Il15ra}^- \text{/Cd11cDr} \) chimeras receiving DT showed an inflammatory skin response similar to that seen in control chimeras receiving PBS (Fig. 2 G). Moreover, Langerin-Dtr \((\text{LangDtr})\) animals treated with DT were indistinguishable from PBS-treated \(\text{LangDtr}\) (Fig. 2 H), suggesting that Langerhans cells were not implicated in IMQ-mediated psoriasiform skin pathology, neither in the IL-15–induced inflammation nor in the IL-15R\( \alpha \)-mediated dampening of disease.

Keratinocytes express high surface IL-15R\( \alpha \) and release IL-15R\( \alpha \) upon stimulation

The aforementioned results suggested that stromal skin cells expressed significant levels of IL-15R\( \alpha \) to dampen IL-15–induced cutaneous inflammation. We assessed IL-15R\( \alpha \) expression by stromal cells dampens psoriasiform inflammation

Similar to the aforementioned experiments, we generated BM chimeras to investigate the cellular source of IL-15R\( \alpha \). Interestingly, selective absence of IL-15R\( \alpha \) on radio-resistant cells in WT–Il15\(^{-/-}\) chimeras nicely replicated the exaggerated inflammatory skin response found in Il15\(^{-/-}\) mice (Fig. 2, E and F). This was not caused by the persistence of radio-resistant skin DCs expressing lower CD11c levels, including Langerhans cells, the latter of which have been shown to resist high-dose irradiation (Merad et al., 2002). Thus, upon IMQ treatment, Il15\(^{na^-/-}\text{Cd11cDr} \) chimeras receiving DT showed an inflammatory skin response similar to that seen in control chimeras receiving PBS (Fig. 2 G). Moreover, Langerin-Dtr \((\text{LangDtr})\) animals treated with DT were indistinguishable from PBS-treated \(\text{LangDtr}\) (Fig. 2 H), suggesting that Langerhans cells were not implicated in IMQ-mediated psoriasiform skin pathology, neither in the IL-15–induced inflammation nor in the IL-15R\( \alpha \)-mediated dampening of disease.
protein levels in single-cell suspensions of murine total skin using flow cytometry. Upon gating on immune lineage-negative (nonimmune) cells, there was a clear population of cells expressing high levels of IL-15Rα, which was present in the epidermal, but not dermal, fraction (Fig. 3 A, left; and not depicted). Strikingly, this IL-15Rα high stromal cell subset was >95% positive for the keratinocyte-specific marker (cyto-) keratin-19 (Fig. 3 A, right). Isotype-matched control staining of WT murine keratinocytes gave only background signals, comparable to IL-15Rα staining of Il15ra−/− murine keratinocytes (Fig. 3 B).

Similar to murine cells, keratin-19+ epidermal cells obtained from healthy human individuals also expressed significant IL-15Rα levels (Fig. 3 C, top), as was the case for keratin-19+ cells from skin plaques of psoriasis patients (Fig. 3 C, bottom). Although keratinocytes from skin plaques of psoriasis patients showed a tendency toward higher expression levels of cell surface IL-15Rα compared with keratinocytes from healthy individuals, upon subtraction of background staining, IL-15Rα levels were not significantly different between these groups (Fig. 3 D).

In primary normal human keratinocyte cultures, stimulation with proinflammatory cytokines, including IL-1β, IFN-α, IFN-γ, and TNF, induced the release of soluble IL-15Rα (sIL-15Rα) into the supernatant (Fig. 4, A and B). Similar results were obtained using primary murine keratinocytes and human keratinocyte cell lines (unpublished data). Interestingly, the secretion of sIL-15Rα by human...
keratinocytes upon stimulation with the aforementioned cytokines was almost absent in the presence of the matrix metalloproteinase (MMP) inhibitor GM6001 (Fig. 4 B). In line with these in vitro data, administration of GM6001 to WT mice receiving topical IMQ exacerbated cutaneous inflammation, in comparison to IMQ-treated WT mice receiving PBS, whereas co-administration of recombinant sIL-15Rα was able to correct the GM6001-mediated exacerbation (Fig. 4 C). Moreover, there was no evidence of increased alternative splicing and generation of IL-15Rα lacking the transmembrane part (Δtm) encoded by exon 6 (Dubois et al., 1999), as shown by reverse transcription polymerase chain reaction using specific primers for full-length and Δtm IL-15Rα (Fig. 4 D).
Collectively, these data show that murine and human keratinocytes express significant levels of IL-15Rα, both during homeostasis and inflammation and that upon stimulation with proinflammatory cytokines, keratinocytes are able to release sIL-15Rα via proteolytic cleavage by MMPs.

**IL-15 drives T cell expansion and production of proinflammatory cytokines**

As previously mentioned, IL-15 is able to induce the production of different proinflammatory cytokines implicated in the pathogenesis of psoriasis (McInnes and Liew, 1998). Moreover, IL-15 is a common γ chain (γc) cytokine and, similar to other γc cytokine members, IL-15 is involved in the survival and maintenance of T cells, especially memory CD8+ and, to a lesser extent, memory CD4+ T cells (Boyard et al., 2012).

Paralleling the degree of psoriasiform inflammation, Il15ra+ animals showed the strongest expansion of CD3+ T cells upon IMQ treatment, followed by WT and Il15ra–/– mice (Fig. 5 A and B). The increase in cell counts and percentages was most notable for CD8+ T cells, which were over 5-fold more prominent in Il15ra+ animals compared with WT and over 20-fold in comparison to Il15ra–/– mice. CD4+ T cells were also increased in IMQ-treated Il15ra+ animals, being twice as abundant as in WT and three times more than in Il15ra–/– mice.

As for effector cytokines, the mRNA levels of IL-15 and TNF, as well as IL-23p19, IL-17, and IL-22, were 3–6-fold higher in Il15ra–/– animals compared with WT mice upon IMQ application for 3 d (Fig. 5 C). Conversely, levels of these cytokines were at least twofold lower in Il15ra+ mice compared with WT (Fig. 5 C).

In line with these expression data, IL-17–producing αβ and γδ T cells were increased in Il15ra–/– mice in comparison to WT, whereas Il15ra+ animals had significantly lower counts of IL-17+ skin T cells (Fig. 5 D and E).

**Administration of sIL-15Rα inhibits human and murine psoriasiform disease**

Our data showing that IL-15Rα derived from stromal cells, most notably from keratinocytes, dampens IMQ-induced skin disease suggested that administration of IL-15Rα molecules might improve psoriasiform inflammation. As murine skin lacks distinct features of human skin, and thus cannot entirely mimic psoriasis, we expanded our findings in the IMQ mouse model by taking advantage of our well-established human xenograft psoriasis model (Boyard et al., 2004). As expected, transplantation of symptomless prepsoriatic skin grafts from a psoriasis patient onto immunodeficient AGR129 mice induced the skin grafts to spontaneously develop a fully-fledged psoriatic phenotype within 6–8 wk. Thus, human xenografts of PBS-treated control animals showed characteristic features of psoriasis, including acanthosis, papillomatosis, loss of granular cell layer, and dense infiltrates of mononuclear cells in the dermis and epidermis representing immune cells (Fig. 6, A and B). In sharp contrast to PBS controls, xenografts of AGR129 mice treated with sIL-15Rα did not develop any typical morphological signs of psoriasis, and, accordingly, the acanthosis and papillomatosis indices remained low (Fig. 6, A and B).

Similar to AGR129 mice, treatment with sIL-15Rα was also efficacious in the IMQ model of psoriasiform disease. Thus, WT animals receiving sIL-15Rα resembled Il15ra–/– mice in that development of cutaneous inflammation and thickening upon IMQ application was significantly blunted, corresponding to only 35% of the increase in thickness compared with WT controls (Fig. 6, C and D). Interestingly, administration of sIL-15Rα to Il15ra+ animals controlled the aberrant skin inflammation in these mice (Fig. 6, C and D).

To gain insight into the target cells of IL-15 and to rule out the possibility that sIL-15Rα associates with soluble IL-15 to form agonistic complexes that stimulate T cells and keratinocytes (Rückert et al., 2000; Rubinstein et al., 2006; Stoklasek et al., 2006), we stimulated primary keratinocytes and T cells using IL-15 without or with addition of sIL-15Rα. Paralleling the aforementioned results, production of IL-1β, IL-6, and TNF by IL-15–stimulated primary human and murine keratinocytes was significantly reduced when sIL-15Rα was added to the cultures (Fig. 6 E; and not depicted). Moreover, culturing of total skin cells with IL-15 led to an increase of IL-17–producing αβ and γδ T cells, which was significantly reduced when sIL-15Rα was co-administered (Fig. 6, F and G). Notably, skin-resident T cells expressed IL-15 receptors, and levels of IL-15RB (CD122) and γδ on these cells did not change upon application of IMQ (unpublished data).

**Opposing regulation of IL-15 and sIL-15Rα in psoriasis patients influences T cell proliferation**

Our data on IL-15Rα expression on human keratinocytes and the role of sIL-15Rα in dampening psoriasiform disease in two relevant animal models suggested that the IL-15–IL-15Rα system might also play an important role in psoriasis patients. To this end, we assessed IL-15 and sIL-15Rα levels in serum of psoriasis patients (n = 52) using ultrasensitive radio-immunooassays. IL-15 levels steadily rose with increasing disease severity, as reflected by the psoriasis area and severity index, following a linear correlation (Fig. 7 A; r = 0.72; Spearman test, P < 0.0001). Strikingly, however, sIL-15Rα showed a completely opposite trend. Thus, serum sIL-15Rα levels decreased with higher PASI scores, leading to a negative linear correlation (Fig. 7 B; r = −0.49; Spearman test, P < 0.0001).

The opposing regulation of IL-15 and sIL-15Rα could indicate interindividual variability. Thus, we measured these parameters in individual psoriasis patients before and after anti-psoriatic treatment (n = 17). As expected, antipsoriatic therapy led to a marked reduction of disease severity in most of the patients as measured by PASI, which was paralleled by a significant decrease of serum IL-15 to background levels in the patients (Fig. 7 C, left and middle). In sharp contrast to IL-15, serum levels of sIL-15Rα rose 2–12-fold after treatment (Fig. 7 C, right).
To assess the biological activity of serum IL-15 and sIL-15Rα, we performed direct ex vivo proliferation assays using the IL-15–sensitive T cell line CTLL-2. Direct culturing of CTLL-2 cells with sera led to proliferation of the cells, which was most prominent when using sera from patients with higher PASI scores (Fig. 7 D). The biological property of sIL-15Rα was assessed by culturing CTLL-2 cells with a fixed concentration of recombinant human IL-15, followed by addition of sera. Samples of psoriasis patients with PASI scores of 5 and lower almost completely inhibited IL-15–mediated proliferation of CTLL-2 cells, whereas serum specimens of patients with increasing PASI scores progressively lost the ability to block IL-15–mediated T cell stimulation (Fig. 7 E).

These data establish in psoriasis patients that disease severity and IL-15 correlate directly with each other, whereas concomitant regulation of biologically active sIL-15Rα follows an opposite trend.

Figure 5. IL-15 drives T cell expansion and production of proinflammatory cytokines. (A and B) WT, Il15−/−, and Il15ra−/− mice were treated for 6 consecutive days with IMQ cream on their right ear. Epidermal skin cells were isolated and analyzed by flow cytometry for T cell subsets. Shown are absolute numbers (A) and dot plots (B) of CD8+ (open bars) and CD4+ (filled bars) CD3+ T cells in indicated animals. (C) mRNA levels of IL-15, IL-15Rα, TNF, IL-23p19, IL-17, and IL-22 were measured by quantitative RT-PCR in ears of WT, Il15−/−, and Il15ra−/− mice treated for 3 consecutive days with IMQ. Shown is fold increase in mRNA normalized to ribosomal protein L27 versus control skin of WT mice. Data are displayed as mean ± SD. (D and E) WT, Il15−/−, and Il15ra−/− mice received IMQ for 3 d, followed by direct ex vivo analysis of intracellular IL-17 production in αβ (D) and γδ T cells (E). Data are pooled results from two to four independent experiments. P-values were determined using one-way ANOVA; *, P < 0.01; **, P < 0.001; ***, P < 0.0001.
Figure 6. Administration of sIL-15Rα inhibits human and murine psoriasiform disease. (A and B) Development of a psoriatic phenotype in symptomless prepsoriatic human skin transplanted onto AGR129 mice is prevented by injection of sIL-15Rα. Shown is representative hematoxylin-eosin staining at low- (left) and high-power (right) magnification (A), and acanthosis (B, top), and papillomatosis index (B, bottom) of the human skin grafts 6 wk after transplantation onto AGR129 mice receiving either PBS or sIL-15Rα. Bars, 50 µm. Data are representative of four mice per group. (C and D) WT, WT receiving sIL-15Rα, and Il15ra−/− mice injected with sIL-15Rα were treated for 6 consecutive days with IMQ cream on their right ear. Mice were assessed as in Fig. 1 (B and C). (E) Primary human keratinocytes were stimulated with either PBS, IL-15, IL-15 plus sIL-15Rα, or sIL-15Rα, followed by analysis of IL-1β, IL-6, and TNF by ELISA. (F and G) Epidermal skin cells from WT mice were stimulated with either PBS, IL-15, IL-15 plus sIL-15Rα, or sIL-15Rα, followed by analysis using flow cytometry. Shown are percentages of IL-17-producing CD3+CD45+ T cells (F) and γδ T cells (G) as mean ± SD. Data are representative of two independent experiments. P-values were determined using one-way ANOVA; *, P < 0.01; **, P < 0.001; ***, P < 0.0001.
DISCUSSION

Our data demonstrate that during inflammation availability of IL-15 is regulated by proteolytic cleavage and shedding of IL-15Rα from epidermal stromal cells, unveiling an unexpected role of tissue-derived sIL-15Rα in psoriasis. Although the crucial function of IL-15Rα in the IL-15 system is well known, the role of endogenous sIL-15Rα in IL-15 homeostasis has remained elusive. Hence, Il15−/− and Il15ra−/− mice both lack memory CD8+ T cells and NK cells (Lodolce et al., 1998; Kennedy et al., 2000; Boyman et al., 2012), implying that IL-15 depends on IL-15Rα for signaling. This is explained by the fact that IL-15 binds cells expressing surface IL-15Rα together with CD122 and γc. In addition to this classical cis interaction, DCs can produce and present IL-15 in trans via membrane-bound IL-15Rα to neighboring CD8+ and NK cells expressing dimeric IL-15Rβγ, consisting of CD122 and γc (Dubois et al., 2002; Schluns et al., 2004; Burkett et al., 2004). Thus, binding and presentation of IL-15 by membrane-bound IL-15Rα delivers a stimulatory signal.

Moreover, IL-15Rα also exists in a soluble form and, unlike membrane-bound IL-15Rα, sIL-15Rα can act either as an agonist or antagonist of IL-15 in vivo. Thus, recombinant sIL-15Rα combined with recombinant IL-15, thereby forming IL-15–sIL-15Rα complexes, can cause massive expansion of memory CD8+ and NK cells in mice (Rubinstein et al., 2006; Stoklasek et al., 2006). Conversely, injection of recombinant sIL-15Rα is able to reduce collagen-induced arthritis (Ruchatz et al., 1998), and to inhibit homeostasis of memory CD8+ and NK cells in vivo (Khan et al., 2002; Nguyen et al., 2002). The implications from these data are that sIL-15Rα is a potent regulator of IL-15 activity, acting as an agonist or antagonist. However, there is no evidence as to whether endogenous, natural (i.e., not recombinant) sIL-15Rα is able to modulate IL-15 signaling in vivo. The data presented in this study demonstrate for the first time that endogenous sIL-15Rα is implicated in reducing cutaneous inflammation in psoriasis, both in mice and men.

We show that during psoriasis inflammation, abundant production of IL-15 by DCs causes the activation of αβ and γδ effector T cells. These T cells need only to express dimeric IL-15Rβγ to efficiently bind IL-15 and signal, as demonstrated by experiments using Il15ra−/−–WT mixed BM chimeras lacking membrane-bound IL-15Rα on both T cells and DCs. Hence, Il15ra−/−–WT chimeras show identical inflammatory responses as WT–WT chimeras. Notably, the dimeric IL-15Rβγ possesses an affinity for IL-15 of ~10−9 M, and thus is able to avidly bind IL-15, even in the absence of IL-15Rα in cis or in trans (Waldmann and Tagaya, 1999). However, sIL-15Rα has an
affinity for IL-15 of ~10^{-11} M, which is 100-fold higher than the dimeric IL-15Rβγ, thus, even small concentrations of sIL-15Rα are able to compete with IL-15 binding to the dimeric IL-15Rβγ. This notion fits well with our herein presented data. Moreover, as demonstrated by using WT-II15α−/− mixed BM chimeras, in the absence of stromal sIL-15Rα, the stimulatory action of IL-15 on αβ and γδ effector T cells becomes unopposed, thus leading to an inflammatory response that is even more pronounced than in WT mice.

These data also highlight the role of IL-15 in driving an (auto-) inflammatory pathology. IL-15 boosts the production of proinflammatory cytokines, including TNF, by keratinocytes and T cells, as well as IL-17, IL-22, and IL-23 by murine αβ and γδ T cells, as shown here, and IL-17 by human T cells (Hoeve et al., 2006). In turn, TNF is a well-known driver of the inflammatory response in psoriasis (Mease et al., 2000; Chaudhari et al., 2001; Boyman et al., 2004) and also enhances the production of other proinflammatory cytokines, including IL-15, thereby fueling a positive feedback loop. Moreover, IL-15 has been suggested to inhibit Fas-induced apoptosis of keratinocytes (Rückert et al., 2000).

In line with other studies showing that psoriasiform inflammation can be inhibited by blocking IL-15 signals using exogenous administration of an anti–IL-15 mAb (Villadsen et al., 2003), we demonstrate that this pathological inflammation can be dampened both in human and murine skin by injecting recombinant sIL-15Rα in the AGR mouse model of psoriasis or to WT and II15α−/− animals in the IMQ model. Remarkably, endogenous sIL-15Rα is able to exert a similar function as recombinant sIL-15Rα by depriving pathogenic T cells from contact with IL-15. In line with these data in mice, we also demonstrate that levels of sIL-15Rα negatively correlate with disease severity in psoriasis patients and that serum concentrations of sIL-15Rα rise upon successful therapy. Using direct ex vivo testing of patients’ sera containing high titers of sIL-15Rα, we found that sIL-15Rα inhibited IL-15-driven T cell proliferation. Likewise, treatment of patients with Crohn’s disease using the anti–TNF mAb infliximab has been shown to increase serum levels of sIL-15Rα, presumably via reverse signaling of infliximab through TNF receptors (Bouchaud et al., 2010), suggesting that sIL-15Rα might serve as an IL-15 antagonist also in inflammatory conditions other than psoriasis. It will be interesting to perform subgroup analyses of larger cohorts of psoriasis patients before and during antipsoriatic treatment to determine whether top-ical treatment is also able to correct the serum levels of sIL-15Rα and whether some systemic therapeutics are more efficient than others in restoring serum sIL-15Rα levels.

An important point of the present study worth emphasizing is that sIL-15Rα is produced and shed by stromal cells, thus contributing to the cross-talk between stroma and immune system. In our hands, IL-15Rα shedding was induced in human and murine keratinocytes upon incubation with different proinflammatory cytokines and was dependent on proteolytic cleavage by MMPs, as demonstrated by using a specific MMP inhibitor. Conversely, alternative splicing of IL-15Rα and production of IL-15Rα molecules lacking the transmembrane domain, was not increased during psoriasiform inflammation. These findings show that inflammation-mediated expression of MMPs provides a negative feedback via the release of sIL-15Rα to dampen IL-15–driven pathology. Moreover, the data illustrate that stromal cells, including keratinocytes, are not only able to amplify inflammation via the production of proinflammatory cytokines and antimicrobial peptides but they also possess the capacity to control an inflammatory response, at least to a certain degree. Thus, in psoriasis patients, serum sIL-15Rα is presumably able to buffer systemic IL-15 levels in mild disease. However, with increasing severity of psoriasis, the rise of IL-15 is not accompanied by a release of sIL-15Rα. This failure to further increase the levels of sIL-15Rα could result from limited bioavailability of stroma-derived sIL-15Rα, perhaps due to exhaustion or from a defect in shedding.

Our study highlights the importance of tissue-specific mechanisms in regulating availability of cytokines by showing the crucial role of the IL-15−/− IL-15Rα system in chronic inflammation. Manipulating IL-15 signaling, by provision of recombinant sIL-15Rα or use of neutralizing antibodies specific for IL-15, holds the promise to interfere with the vicious cycle driving chronic inflammatory disorders, including psoriasis, inflammatory bowel disease, and rheumatoid arthritis (Baslund et al., 2005).

MATERIALS AND METHODS

Mice and human subjects. C57BL/6 (WT) and II15α−/− mice were purchased from The Jackson Laboratory, and IL-15−/− animals were from Taconic. Mice expressing DT receptor (DTR) under control of the CD11c (Cd11cDtr) or the Langern promoter (LangDtr) have been published previously (Jung et al., 2002; Kissenpfennig et al., 2005) and were purchased from The Jackson Laboratory or provided by B. Malissen (Centre d’Immunologie de Marseille-Luminy, Marseille, France), respectively. All transgenic mice were on a C57BL/6 background. For depletion of CD11c+ cells in CD11cDtr or Langern+ cells in LangDtr mice, animals received intraperitoneal injections of 200 ng DT (Sigma-Aldrich) in PBS every other day, starting 1 d before IMQ treatment. AGR129 mice deficient in type I and II IFN receptors in addition to being Rag2−/− have been previously published (Boymann et al., 2004). Mice were maintained under specific pathogen-free conditions and used at 6–8 wk of age. Animal experiments were performed in accordance with Swiss Federal Veterinary Office guidelines and approved by the Cantonal Veterinary Office in Zurich (Veterinäramt, Gesundheitsdirektion Kanton Zürich). Use of human samples was approved by the local ethical committee and informed consent was obtained from patients. 17 patients were recruited and seen before and after antipsoriatic treatment. The patients’ mean age was 44.2 (±12.6) years; and the male/female ratio was 14:3; mean disease duration was 9.8 yr; 4 out of 17 patients had evidence of psoriatic arthritis; and on average patients had previously received 1–2 systemic treatments (range, 1 to 5). During this study, patients examined before and on treatment were administrated systemic fumaric acid ester, systemic methotrexate, systemic TNF-blocking agents, or systemic p40 IL-12/-23-blocking agents.

IMQ-induced psoriasiform skin inflammation model. For induction of skin inflammation, mouse ears or shaved backs were treated for 6 consecutive days using 60–80 mg Aldara cream containing 5% (3–4 mg) IMQ (3M Pharmaceuticals) or Vaseline (Vifor SA). Mice were evaluated daily by measuring ear thickness using a digital micrometer (Mitutoyo). For treatment using sIL-15Rα, mice received five consecutive intraperitoneal injections of 3 µg soluble...
human IL-15Rα-Fc (R&D Systems). The MMP inhibitor GM6001 (Merck) was given by intraperitoneal injections at 250 µg every day for 6 d.

**AGR129 human xenograft psoriasis model.** Transplantation of symptomless psoriatic skin grafts from psoriasis patients onto immunodeficient AGR129 mice was performed as previously described (Boyan et al., 2004), leading to the spontaneous development of a fully-fledged psoriatic phenotype within 6–8 wk.

**Histopathological and immunohistochemical analysis.** Mice and human skin samples were snap frozen and stained using hematoxylin-eosin or immunohistochemistry, as previously reported (Boyan et al., 2004). Epidermal thickness (termed acanthosis for human skin samples) and the ratio of maximal/minimal epidermal thickness (termed papillomatosis for human skin samples) were evaluated for at least 4 mice, as previously reported (Boyan et al., 2004). In murine skin samples, epidermal thickness was determined by measuring the thickness of the interfollicular epidermis at 10 representative sites of the histological slide. In human skin samples, acanthosis was obtained by measuring the thickness of the viable epidermis at 10 representative sites of the histological slide showing an uninterrupted epidermis. Sections were analyzed using ImageScope for image acquisition (Aperio Technologies, Inc.).

**Quantification of cytokine levels.** Quantification of sIL-15Rα and IL-15 was determined, as previously established (Bouchaud et al., 2010). Sandwich radio-immunoassays were set up using goat anti-human IL-15Rα or mouse anti-human IL-15 mAbs (R&D Systems) as capture and radio-labeled anti-human IL-15Rα (R&D Systems) or anti-human IL-15 mAbs (Diakone) as tracer. Recombinant human IL-15Rα (R&D Systems) and recombinant human IL-15 (eBioscience) served as standards. Supernatants of each well were collected, and the wells were washed twice with PBS. The radioactivity associated with the wells (bound fraction) or contained in the supernatants and washes (unbound fraction) was determined. The quantitative measurement of IL-1β, IL-6, and TNF was performed using commercially available solid-phase ELISA kits (R&D Systems). All samples were assayed in triplicates.

**Keratinocyte cultures.** Primary human keratinocytes isolated from foreskin were cultured in 6-well plates using Keratinocyte-SFM media (Gibco) plus epidermal growth factor (5 ng/ml) and bovine pituitary extract (50 µg/ml) at 33°C. Primary keratinocytes or the human keratinocyte cell line HaCaT were used for obtaining single-cell suspensions from BM of murine tibias and femurs, followed by culturing the cells in DC media containing RPMI supplemented with 5% FCS and nonessential amino acids, 2 mM l-glutamine, and 100 U/ml penicillin/streptomycin for 1 d. Subsequently, nonadherent cells were washed twice with DC media supplemented with GM-CSF (R&D Systems) for another 5 d, followed by maturation of cells (using LPS overnight), harvesting, and intradermal injection into the ear of 1 × 10^6 cells.

**Flow cytometry.** For obtaining single-cell suspensions, mouse or human skin was incubated in dispase (5 mg/ml) to separate epidermis from dermis. Epidermal cell suspensions were prepared by treating epidermis with trypsin-EDTA. Collagenase IV, DNase I, and trypsin-EDTA were used to obtain dermal suspensions. Single-cell suspensions were stained according to standard protocols using PBs containing 1% FCS and 2 mM EDTA (Krieg et al., 2010), with the following fluorochrome-conjugated mAbs (BD, unless otherwise stated): anti-mouse αβ TCR, γδ TCR, CD3, CD4, CD8, CD11b, CD11c, CD45.2, CD207, (cyto-) keratin-19 (Dako), IL-15Rα, IL-17, and MHC class II; anti-human CD3, CD11b, CD11c, CD207, MHC class II, and anti-human IL-15Rα and (cyto-) keratin-19 (both from eBioscience). For intracellular IL-17 staining, mice received IMQ for 3 d, followed by intravenous injection of 250 µg of Brefeldin A (Sigma-Aldrich) 4 h before euthanization and analysis by flow cytometry. Alternatively, mouse cells were isolated and stimulated in vitro with 100 ng/ml recombinant human IL-15 without or with sIL-15Rα-Fc for 24 h in the presence of Golgi stop (BD). After cell surface staining, cells were fixed, permeabilized, and stained intracellularly for IL-17. Samples were harvested using a FACSCanto II (BD) and analyzed using FlowJo software (Tree Star).

**RT-PCR.** Ears were immersed in mRNALater solution (Applied Biosystems) and kept at −80°C, and then homogenized using Tissue Lyser II (QIAGEN) and total mRNA was purified using the Fibrous Minui kit (QIAGEN). After reverse transcription into cDNA using the Reverse Transcription kit (QIAGEN), PCR was performed on a Viia7 RT-PCR detection system (Applied Biosystems). Full-length versus Δtm Il15sα mRNA was assessed using RT-PCR, whereas levels of all other cytokines were determined via quantitative RT-PCR using SYBR Green supermix (QIAGEN). The sequences of specific primers (from Microsynth) were as follows: Il15 forward, 5’-CATCCATCTCGTGACTCATGTGTTG-3’, Il15 reverse, 5’-GCCCTCT-GTTTATGGGACACCT-3’; full-length Il15a forward, 5’-ATGCGCT-GCGCGACAG-3’; full-length Il15a reverse, 5’-TATGGCTCTGCTGTCTCATACC-3’; Δtm Il15a forward, 5’-ATGCGCTCGCCGACAG-3’; Δtm Il15a reverse, 5’-GAACCTTGATGACCTGCTGC-3’; B77 forward, 5’-TTGGAGGCACAGACCCACC-3’; B77 reverse, 5’-GATAGCCGTCCTC-3’; B122 forward, 5’-AGCGGACATCTGTGTTGTATAC-3’; B122 reverse, 5’-CTGGCACCACCTGGTTGT-3’. Gene expression was normalized using the ribosomal protein L27 (RPL27) housekeeping gene and data are reported as fold differences by the 2^(-ΔΔCt) method, with ΔCt = Ct_target gene − Ct.housekeeping gene, and ΔΔCt = ΔCt_sample − ΔCt_reference as previously published (Cai et al., 2011).

**Statistical analysis.** Statistical analyses were done using one-way ANOVA with Bonferroni’s multiple comparison test, a Mann-Whitney U test, or a Spearman test, as indicated.

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