IL-23-Mediated Epidermal Hyperplasia Is Dependent on IL-6

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Psoriasis is a chronic inflammatory skin disease primarily driven by Th17 cells. IL-23 facilitates the differentiation and induces complete maturation of Th17 cells. Lesional psoriatic skin has increased levels of IL-23 and recent studies show that intradermal injections of IL-23 induce a psoriasis-like skin phenotype in mice. We have now characterized the IL-23-induced skin inflammation in mice at the molecular level and found a significant correlation with the gene expression profile from lesional psoriatic skin. As observed in psoriasis, the pathogenesis of the IL-23-induced skin inflammation in mice is driven by Th17 cells. We demonstrate a dramatic upregulation of IL-6 mRNA and protein after intradermal injections of IL-23 in mice. Using IL-6$^{-/-}$ mice we show that IL-6 is essential for development of the IL-23-elicited responses. Despite producing high levels of IL-22, IL-6$^{-/-}$ mice were unable to express the high-affinity IL-22 receptor chain and produced minimal IL-17A in response to intradermal injections of IL-23. In conclusion, we provide evidence for the critical role played by IL-6 in IL-23-induced skin inflammation and show that IL-6 is required for expression of IL-22R1A.

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INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease (Nickoloff *et al.*, 2007; Sabat *et al.*, 2007). Histological features of psoriatic skin include epidermal hyperplasia, increased angiogenesis, and leukocyte infiltration where CD4$^+$ (CD4 positive cell) T cells predominate in the dermis and CD8$^+$ T cells infiltrate the epidermis (Bowcock and Krueger, 2005; Danilenko, 2008). In psoriatic skin, the differentiation process is drastically shortened (Sabat *et al.*, 2007), indicating disturbed keratinocyte (KC) differentiation, which together with infiltrating T cells constitute key pathological characteristics of psoriasis (Lowes *et al.*, 2007).

One sub-population of CD4$^+$ T cells, termed Th17 cells has recently been shown to be pivotal players in psoriasis (Lowes *et al.*, 2008; Kagami *et al.*, 2010). Th17 cell differentiation from naïve CD4 T cells is induced by the presence of transforming growth factor-β and IL-6 (or IL-21), which upregulates the transcription factor ROR-gt. This event triggers the expression of IL-23R, which when bound to IL-23, serves to expand and stabilize Th17 responses. The combination of transforming growth factor-β and IL-6 suppresses the expression of the transcription factor FOXP3, preventing differentiation of naïve CD4 T cells to regulatory T cells (Tregs). IL-6 specifically has a critical role in dictating whether an immune response is dominated by FOXP3$^+$ regulatory T cells or Th17 cells as shown previously by generation of antigen-specific regulatory T cells and by inhibition of effector T-cell development in IL-6$^{-/-}$ mice (Korn *et al.*, 2007). On binding of IL-23 to its receptor, Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling is activated leading to transcription of pro-inflammatory cytokines, such as IL-22, IL-17A, IL-17F, and IFN-G (Parham *et al.*, 2002; Di *et al.*, 2009).

IL-23 is overexpressed in psoriatic lesions (Lee *et al.*, 2004; Wilson *et al.*, 2007) and it has recently been shown that intradermal injections of IL-23 in mice provoke a skin phenotype resembling psoriasis. Specifically, injections of IL-23 upregulates pro-inflammatory cytokines and induces KC proliferation leading to epidermal hyperplasia (Kopp *et al.*, 2003; Chan *et al.*, 2006; Zheng *et al.*, 2007; Hedrick *et al.*, 2009). In the present study, we demonstrate a significant correlation between the gene expression profiles of IL-23-induced skin inflammation in mice and of lesional psoriatic skin. Furthermore, we provide evidence for requirement of IL-6 in the development of IL-23-induced skin inflammation. Finally, we elucidate the mechanism behind the decreased skin inflammation in IL-6$^{-/-}$ mice, despite the high expression of IL-22, by showing an impaired expression of the high affinity IL-22 receptor chain and a decreased IL-17A production in response to intradermal injections of IL-23.

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Abbreviations: CD4$^+$, CD4 positive cell; CD8$^+$, CD8 positive cell; JAK, Janus kinase; Ki67, Kiel clone 67; KO, knockout; STAT, signal transducer and activator of transcription; Th1, T helper 1 cells; Th2, T helper 2 cells; Th17, T helper 17 cells; Treg, regulatory T cell; WT, wild type

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RESULTS

IL-23-induced inflammation and epidermal hyperplasia

As previously reported (Zheng *et al.*, 2007; Hedrick *et al.*, 2009), ear swelling increased more than 2-fold after 10 days with intradermal injections of IL-23 in the mouse ear. Additionally, cumulative ear swelling was significantly augmented for IL-23-injected mice compared with control mice. Histological evaluation revealed features of a psoriasis-like phenotype including epidermal hyperplasia, parakeratosis, and dermal infiltration in the IL-23-injected mice. Furthermore, immunohistochemical staining for Ki67 showed a marked increase in proliferative KCs in the basal layer of the epidermis (Supplementary Figure S1a–d online).

Differences in infiltrating cells between skin and secondary lymphoid organs

To identify the phenotype of the immune cells that infiltrated the ear tissue and the draining lymph nodes following injections of IL-23, single-cell suspensions were prepared and analyzed by flow cytometry. In the ear tissue, $CD4^+$ T cells ($CD4^+$ $CD3^+$) showed the most pronounced increase, more than 3-fold, whereas the increase in $CD8^+$ T cells ($CD8^+$ $CD3^+$), macrophages (MHC II^+ $CD11b^+$), dendritic cells (MHC II^+ $CD11c^+$), and neutrophils ($Gr1^{high}$ $CD11b^{high}$ $CD11c^{low}$) increased 2- to 3-fold. In contrast, the draining lymph nodes showed reduced levels of mononuclear cells (particularly neutrophils) after injection of IL-23 compared with control mice (Supplementary Table S1 online). No differences in cellular content in the blood were detected between IL-23-injected mice and controls (data not shown).

The IL-23-induced gene expression in mice correlates significantly with a psoriasis gene expression signature

A meta-analysis was performed based on three published microarray studies, including 90 patients in total, comparing lesional versus nonlesional psoriatic skin (Yao *et al.*, 2008; Nair *et al.*, 2009; Zibert *et al.*, 2010) – see Supplementary methods online. Among the genes identified from the meta-analysis as highly associated with psoriasis (family-wise error rate <0.001), we selected 51 genes whose involvement in psoriasis is well described (Table 1). For a subset of these genes (18 out of 51), we verified that the fold changes calculated from the meta-analysis correlated with the gene expression changes measured using quantitative PCR (qPCR) on lesional versus nonlesional psoriatic skin pooled from five patients ($r=0.74$, $P<0.001$, see Table 1 and Supplementary Figure S3A online). One gene (*IL-20*) is markedly more induced compared with the microarray data. Three genes (*IL-6*, *TNF-α*, and *IL-12B*), which were found to be slightly but very significantly induced on average in the meta-analysis were not verified by qPCR likely due to the small number of patient samples included. Still, for 15 out of 18 genes (83%), the direction of the fold change was in agreement with the microarray data.

Next, we tested our psoriasis gene expression signature against the expression changes found in IL-23-injected mice compared with control mice. Here, 45 out of 51 genes (88%) had fold changes whose direction was in agreement with the psoriasis gene expression signature ($P<0.001$ using Fisher's exact test; see Table 1 and Supplementary Figure 3B online). Among those six genes not in agreement with the psoriasis gene expression signature, only one of them, *Il-20*, was downregulated more than 2-fold. Four genes (*Il-6*, *Il-1b*, *Il-17a*, and *Il-22*) were markedly more induced by the IL-23-induced skin inflammation in mice compared with the psoriasis gene expression signature. In addition, we included the two genes *Defb3 and Defb4* encoding defensins in our qPCR analysis. These genes were not included on the microarray but have been demonstrated to be highly induced in lesional psoriatic skin. High-expression levels of defensins were indeed observed in the IL-23-injected mice as well as in the psoriasis biopsies.

The gene expression profile indicates a very strong Th17 signature as well as a Th1 signature following IL-23-induced skin inflammation in mice. Epidermal differentiation markers (*Lor* and *Flg*) were downregulated, whereas genes associated with KC activation were upregulated in IL-23-injected mice. Overall, the IL-23-induced gene expression profile in mice correlated significantly with the psoriasis gene expression signature ($r=0.45$, $P=0.001$).

Cytokine expression from IL-23-induced skin inflammation in mice correlates with psoriatic cytokine expression

Next, we investigated the cytokine protein levels in ear biopsies after repeated injections of IL-23. The pro-inflammatory cytokines analyzed were all substantially upregulated in the IL-23-injected mice as compared with control mice (Figure 1), whereas the Th2-associated cytokines IL-4 and IL-5 were induced at a very low level (barely above detection limit). Furthermore, signalling downstream of the IL-23 receptor engage the JAK/STAT signalling cascade. Therefore, the expression of STAT3 was also analyzed and our results showed a distinct upregulation of both the inactive and active form of STAT3 in IL-23-injected mice (Supplementary Figure S2 online).

IL-6 has a critical role in IL-23-induced skin inflammation

IL-6 was dramatically upregulated at both the mRNA and protein level following intradermal injections of IL-23 in mice. As IL-6 has a central role in the control of the Th17/Treg balance, we wanted to investigate the importance of IL-6 for the IL-23-induced skin inflammation. IL-23 was injected in $IL\text{-}6^{-/-}$ mice and compared with wild-type (WT) mice (Figure 2a). Interestingly, $IL\text{-}6^{-/-}$ mice had reduced ear swelling following injections of IL-23, diminished by 59% compared with WT mice (Figure 2b). No significant increase in epidermal thickness (Figure 2c) or sustained inflammatory cell infiltration (Figure 2g) could be observed in the $IL\text{-}6^{-/-}$ mice compared with WT controls, in the presence of exogenous IL-23.

The cytokines IL-1B, IL-2, IL-5, IL-10, and tumor necrosis factor-α (Figure 3a) as well as IL-17A (Figure 3b) were expressed at significantly lower levels in $IL\text{-}6^{-/-}$ mice compared with WT mice following injections of IL-23. In contrast, the levels of IL-22 were 2-fold higher in $IL\text{-}6^{-/-}$ mice

Table 1. The IL-23-induced gene expression in mice correlates significantly with a psoriasis gene expression signature

Gene symbol	Psoriasis meta-log2-fc[1]	Psoriasis meta-*P* value	Psoriasis PP versus PN (qPCR) log2-fc[2]	IL-23-injected mice versus control mice log2-fc[3]
Epidermal differentiation				
FLG	−0.75	2E-10		−1.81
LOR	−1.14	3E-12	−2.31	−2.40
Keratinocyte activation				
KRT6A	2.27	7E-17		2.91
KRT16	4.57	1E-16	4.95	2.86
GJB2	2.85	1E-17	3.86	1.98
GJB6	1.76	1E-17	2.86	1.25
Innate immune defense				
CAMP	0.29	6E-10		0.78
DEFB3			2.09	3.60
DEFB4			3.91	2.51
S100A7	0.92	3E-17		3.41
S100A9	4.97	4E-17	5.52	5.64
S100A11	0.56	1E-13		0.31
Keratinocyte proliferation				
MKI67	0.89	3E-15	1.82	0.11
PCNA	0.69	4E-16	0.22	−0.21
Apoptosis				
BAX	0.30	5E-12		0.54
BCL2	−0.23	6E-14		−0.69
Th1 pathway				
IL-12B	0.24	3E-13	−2.74	0.99
IFNG	0.37	8E-17	2.98	2.44
CXCL9	2.46	9E-17		1.82
CXCL10	2.60	1E-16		2.91
IL-18	−0.49	3E-09	−1.33	−1.36
Th17 pathway				
IL-6	0.34	9E-12	−0.48	5.41
IL-17A	0.18	2E-14	1.57	6.89
IL-22	0.17	1E-12	1.03	5.70
IL-1B	0.99	4E-17	3.05	5.41
Inflammation				
ANGPTL4	1.00	1E-16	2.19	1.66
CCL19	1.11	3E-11		1.29
CCL2	1.46	9E-16		2.54
CCL22	1.13	4E-16		−0.40
CCR2	0.38	6E-13		2.17
CD69	0.64	7E-14		0.84
FCER1G	0.52	4E-11		1.95
FGF2	−0.18	5E-08		−0.73
HIF1A	0.52	6E-16		1.39
HMOX1	1.11	1E-14		1.97
ICOS	0.42	2E-15		1.81
IL-19	1.70	4E-15	3.60	4.10
IL-20	1.03	6E-17	6.37	−1.35
IL-4R	1.06	4E-17		3.07
KLK7	0.57	5E-12		−0.49
MMP9	1.59	2E-16		2.72
NFKB1	0.55	7E-16		0.23
PTPRC	0.71	8E-16		1.38
REL	0.53	5E-15		0.60
SOCS1	0.15	2E-11		0.82
SOCS3	0.21	2E-13		3.06
STAT1	1.79	4E-17		1.51
STAT3	0.99	2E-16		0.72
STAT5B	−0.17	2E-11		0.00
TLR2	0.97	6E-17		1.32
TLR7	0.20	5E-11		−0.20
TNF	0.20	2E-12	−0.09	0.82
TSLP	0.32	3E-10		−0.66

Abbreviation: fc, fold change.
[1]Meta-analysis of three published microarray studies (n=90 patients in total) comparing lesional (PP) versus nonlesional (PN) psoriatic skin.
[2]Verification of microarray data by quantitative PCR (qPCR) on lesional versus nonlesional psoriatic skin pooled from 5 patients.
[3]qPCR on IL-23-injected mice versus control mice (n=5 in each group). Log2 fold changes are calculated for each analysis. Weighted log2 fold changes and *P*-values are listed for the meta-analysis.

compared with WT mice injected with IL-23 (Figure 3a). No significant difference in the production of IL-4 and IL-8 was observed between IL-6$^{-/-}$ mice and WT mice.

Expression of the high affinity IL-22 receptor chain IL-22R1 requires IL-6

A previous study has suggested that the Th17 cytokine IL-22 mediates IL-23-induced skin inflammation and acanthosis (Zheng *et al.*, 2007). IL-22 executes its function through the IL-22 receptor complex composed of IL-22R1A and IL-10R2. We now show that although WT mice constitutively expressed and upregulated the IL-22R1A during the

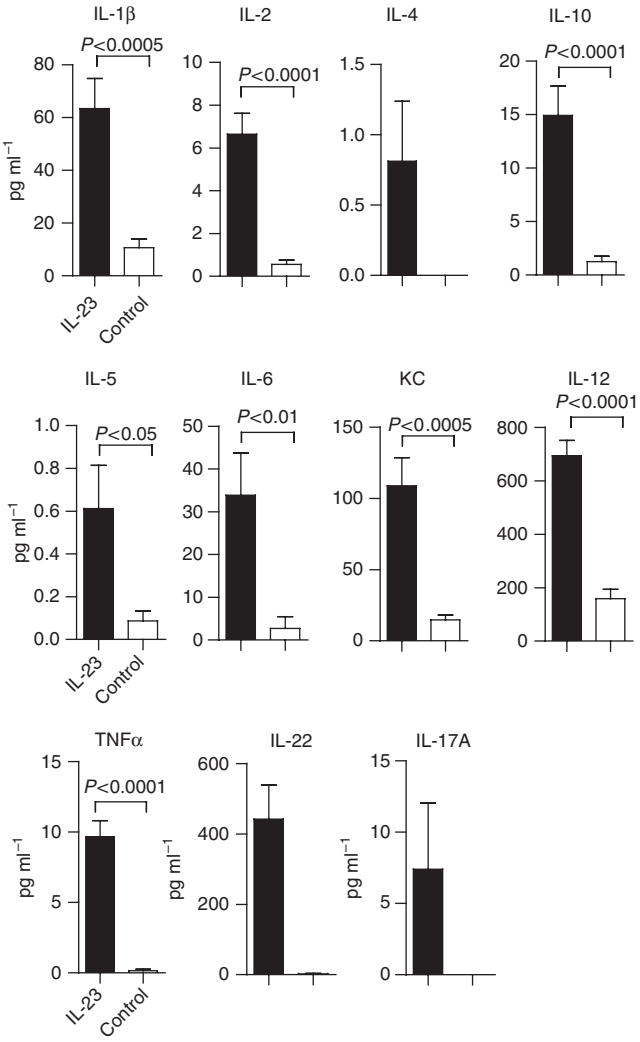


Figure 1. Cytokine expression was upregulated in IL-23-injected WT mice compared with control mice creating an inflammatory Th17 signature similar to that seen in psoriatic patients. Tissue lysates were prepared from frozen ear biopsies and cytokine concentrations were measured by meso scale discovery ($n=10$), except for IL-17A and IL-22, which were measured by ELISA ($n=5$). Mean and ± SEM is indicated for all cytokines. No *P*-values are shown for IL-17A and IL-22 as control groups had levels below the detection limits of the kits.

inflammatory response, the level of IL-22R1A was dramatically lower in IL-6$^{-/-}$ mice (Figure 4a and b). Additionally, a soluble single chain IL-22 binding protein (IL-22BP) has been identified, which sequesters IL-22 and thereby blocks the ligand-induced signalling *in vivo* (Weiss *et al.*, 2004; Wolk *et al.*, 2010). Like the IL-22R1, the IL-22BP was expressed at much lower levels in IL-6$^{-/-}$ mice compared with WT mice after intradermal injections of IL-23 (Figure 4a). These data strongly suggest that IL-6 regulates the expression of the IL-22R1 and the soluble IL-22BP.

IL-23-induced skin inflammation is abrogated by known anti-psoriatic drugs

Systemic daily administration of 20 mg kg^{-1} cyclosporine A (CsA) or 2 mg kg^{-1} Dexamethasone significantly reduced the ear thickness in the IL-23-injected mice. Dexamethasone completely abrogated the inflammatory response, whereas an effect of CsA first appeared at day 4–6 (Figure 2d and e). The CsA-induced response was similar to the profile obtained after injections of IL-23 in IL-6$^{-/-}$ mice (Figure 2a and b) indicating an initial T-cell-independent phase of ear swelling. Histological evaluation revealed a dramatic effect of each treatment on epidermal hyperplasia (Figure 2f). Furthermore, treatment with CsA and Dexamethasone resulted in a marked reduction of *Il-6* and *Il-1b* gene expression levels further underlining the similarity to the response in the IL-6$^{-/-}$ mice (data not shown).

DISCUSSION

Previous studies have described some aspects of the psoriasis-like phenotype following injections of IL-23 in mouse skin (Kopp *et al.*, 2003; Chan *et al.*, 2006; Zheng *et al.*, 2007; Hedrick *et al.*, 2009). In our studies, one of the most striking similarities to psoriasis was epidermal hyperplasia caused by KC hyper-proliferation. This correlates with studies of psoriatic skin showing increased KC proliferation (Sabat *et al.*, 2007). Activation of KCs was evident on injections of IL-23 by upregulation of genes, such as keratin 6 and keratin 16 (*Krt6* & *Krt16*), as we and others have observed in lesional psoriatic skin (Zhou *et al.*, 2003; Gudjonsson *et al.*, 2010). Furthermore, genes associated with epidermal differentiation such as loricrin (*LOR*) and filaggrin (*FLG*), which are downregulated in psoriatic lesions (Watanabe *et al.*, 1991; Mommers *et al.*, 2000; Giardina *et al.*, 2006) and Table 1, are also downregulated during IL-23-induced skin inflammation in mice.

A recent study suggests that IL-23-induced inflammation in mouse skin can be divided into two distinct phases in which the initial ear swelling is T-cell independent followed by a later phase dependent on a Th17 response (Hedrick *et al.*, 2009). Indeed, our data demonstrate cytokine upregulation in the IL-23-injected mice creating an inflammatory Th17 signature similar to that seen in psoriatic patients with high levels of IL-17A and IL-22 as well as tumor necrosis factor-α, IL-1B, IL-8, and IL-6 (Lowes *et al.*, 2007; Nickoloff *et al.*, 2007; Figure 1 and Table 1).

Production of IL-17A and IL-22, induced by injections of IL-23, resulted in a strong upregulation of the innate immune defense genes *Defb3*, *Defb4*, *S100a7*, and *S100a9* (Table 1) as previously described for IL-17A and IL-22 in human KC monolayer cultures and in skin equivalents (Wolk *et al.*, 2004, 2006; Boniface *et al.*, 2005a; Nograles *et al.*, 2008; Guilloteau *et al.*, 2010). Furthermore, a predominantly IL-22-mediated downregulation of epidermal differentiation markers (*KRT10*, *FLG* and *LOR*) similar to the one we observe on injections of IL-23 in mice, has been demonstrated in human KC monolayer cultures and in skin equivalents (Boniface *et al.*, 2005b; Wolk *et al.*, 2006; Nograles *et al.*, 2008).

In our studies, IL-6 was one of the most upregulated cytokines both at the mRNA and protein level following injections of IL-23 in mouse skin. In lesional psoriatic skin, IL-6 is also markedly upregulated at the protein level and slightly, but very significantly, upregulated at the mRNA level

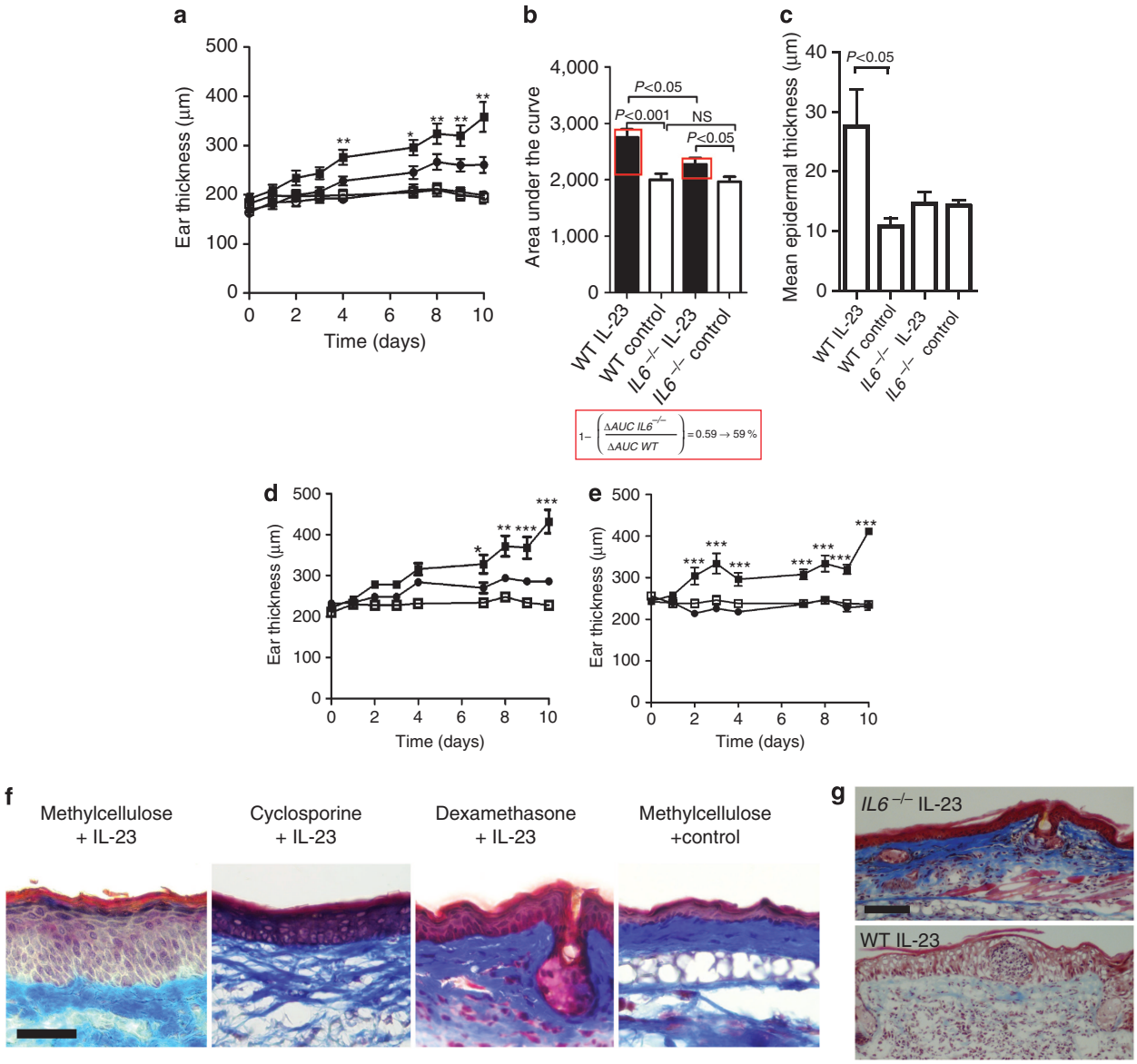
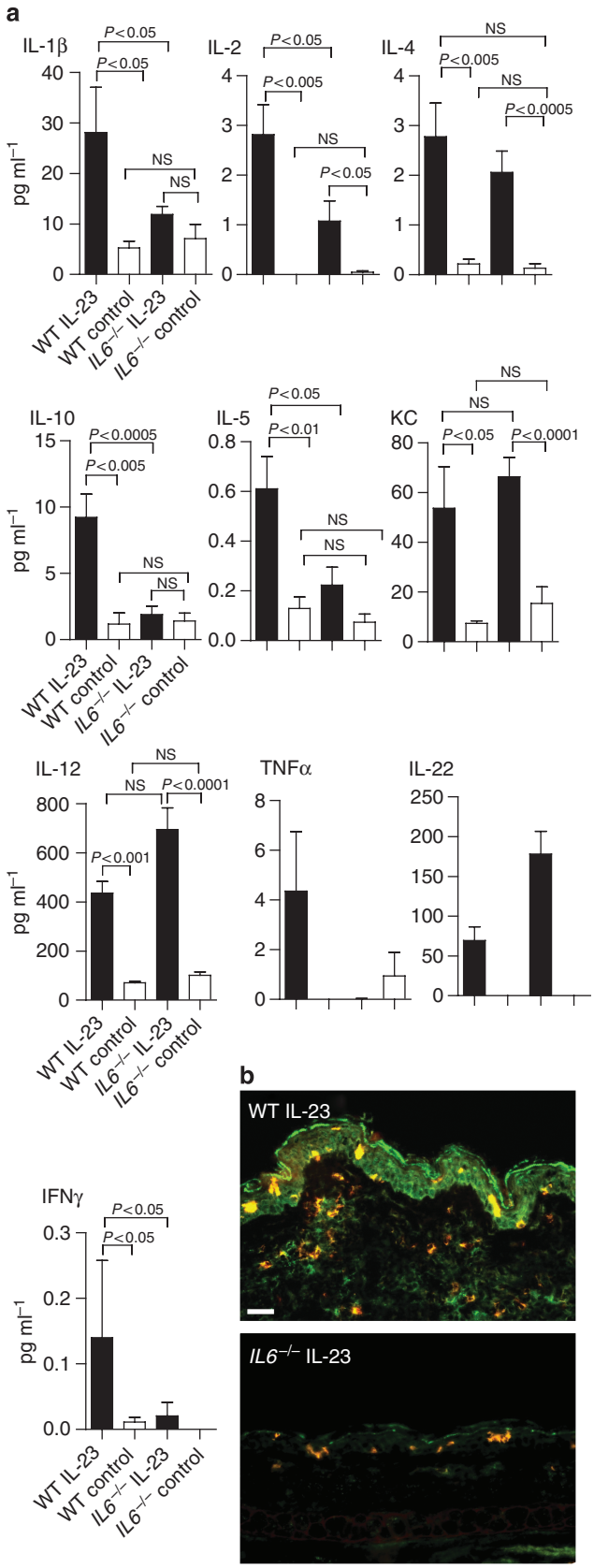


Figure 2. IL-23-induced mouse dermatitis is dependent on IL-6 and can be abrogated by administration of anti-psoriatic drugs. IL-6$^{-/-}$ and WT mice were injected bi-daily intradermally in the ear. (**a**) IL-6$^{-/-}$ + IL-23 (●) or IL-6$^{-/-}$ controls (○) and WT + IL-23 (■) or WT controls (□). *P<0.05, **P<0.01, and ***P<0.001, n = 10–15, (**b**) area under the curve (AUC) ± SEM for IL-6$^{-/-}$ and WT. (**c**) Mean epidermal thickness ± SEM. (**d**, **e**) WT + IL-23 and treated systemically with cyclosporine A (CsA) (**d**) or dexamethasone (**e**), n = 5. (**d**) WT + IL-23 (■), WT + IL-23 + CsA (●), or WT control (□). (**e**) WT + IL-23 (■), WT + IL-23 + dexamethasone (●), or WT control (□). (**f**) Representative Masson's trichrome-stained sections of ear tissue for different treatments as described (bar = 30 μm). (**g**) As in **f** for IL-6$^{-/-}$ (bar = 40 μm).

(Grossman *et al.*, 1989; Neuner *et al.*, 1991; Goodman *et al.*, 2009; Table 1). IL-6 is critically involved in differentiation of naïve T cells to Th17 cells and has recently been proposed to dampen normal regulatory T-cell function, thereby enhancing Th17 driven effector function and sustaining chronic inflammation in psoriasis (Goodman *et al.*, 2009). We therefore investigated the IL-23-induced skin inflammation in IL-6$^{-/-}$ mice, previously shown to be unable to develop Th17 cells, at least *in vitro* (Zheng *et al.*, 2007). Like WT mice, IL-23-injected IL-6$^{-/-}$ mice showed an initial increase in ear thickness. In contrast to WT mice, the ear swelling was not accompanied by infiltration of inflammatory cells, epidermal hyperplasia, or production of pro-inflammatory cytokines during the later time points of the study (i.e., days 6–10). The diminished inflammation during the latter part of the response is likely where T cells are required for modulating the inflammatory response. Indeed, when the calcineurin inhibitor CsA, which inhibits T-cell activation through blocking the transcription factor NFAT (Gottlieb *et al.*, 1992; Menter *et al.*, 2009) was administered to IL-23-injected WT mice a similar response was observed. This result correlates with a previous study (Hedrick *et al.*, 2009), where Rag1^{tm1Mom}-deficient mice were injected intradermally with IL-23. In these mice, an initial T-cell-independent



ear thickening accompanied by a non-T cell derived elevation of IL-22 was observed. Still, the response was not sustained beyond day 5 and was not accompanied by an increase in IL-17A cytokine levels.

Surprisingly, IL-23-injected IL-6$^{-/-}$ mice showed markedly upregulated IL-22 protein levels compared with WT mice. These data partially correlate with those of a previous study (Zheng *et al.*, 2007), where sustained or marginally enhanced IL-22 production was detected in IL-6$^{-/-}$ naïve T cells compared with WT naïve T cells following *in vitro* culture with IL-23. Alternative non-αβ-T-cell sources of IL-22 are likely γδ-T cells (Martin *et al.*, 2009; Siegemund *et al.*, 2009), dendritic cells (Zheng *et al.*, 2007), NK cells (Wolk *et al.*, 2002), and NK T cells, which have been shown to produce IL-22 and express the IL-23R (Kastelein *et al.*, 2007; Goto *et al.*, 2009). Additionally, treatment of NK-T cells with IL-6/transforming growth factor-β was shown to reduce the production of IL-22 (Goto *et al.*, 2009). Thus, in IL-6$^{-/-}$ mice *in vivo* IL-22 is produced directly by naive T cells in response to IL-23 and is likely also produced by NK, NK-T and γδ-T cells. Collectively, these cell subsets may compensate for lack of Th17-derived IL-22 production, but is nevertheless not able to sustain dermal inflammation during the later T-cell-dependent phase of the response.

IL-22 signals through the IL-22 receptor complex composed of the IL-22R1 and the IL-10R2 subunits. In our studies, we observed a disability in IL-6-deficient animals to express the IL-22R1 as well as the regulatory soluble single-chain receptor IL-22BP. This strongly suggests that IL-6 is required for the functional effect of IL-22 and explains the lack of sustained epidermal inflammation and acanthosis in IL-6$^{-/-}$ mice following intradermal injections of IL-23.

Interestingly, and in contrast to a previous report (Hedrick *et al.*, 2009), IL-10 was markedly upregulated at both the mRNA and protein level following injections of IL-23 in WT mice. This was surprising as IL-10 primarily is associated with regulatory T-cell function and an anti-inflammatory response (Sato *et al.*, 1999; O'Garra *et al.*, 2004). However, IL-6 has been shown to directly induce IL-10 production from naïve CD4^{+} T cells as well as under Th17 polarizing conditions (McGeachy *et al.*, 2007). We observed no or marginal IL-10 production following injections of IL-23 in IL-6$^{-/-}$ mice indicating that IL-6 is required for the observed production of IL-10.

IL-19 and IL-24 have been debated in recent studies for their presence and sufficiency in mediating epidermal

Figure 3. Deficiency of IL-6 dramatically influences the cytokine profile in IL-23-injected ears. (**a**) Tissue lysates were prepared from frozen ear biopsies and cytokine concentrations were measured by meso scale discovery ($n = 5$–10), except for IL-22, which were measured by ELISA ($n = 5$–10). Mean ± SEM is indicated for all cytokines. No *P*-values are shown for IL-22 as control groups had levels below the detection limits of the kits. (**b**) Representative double immunofluorescent stainings for CD3ε (red) and IL-17A (green) in WT and IL-6$^{-/-}$ mouse ear tissue, respectively, following intradermal injection of IL-23 ($n = 5$). Co-localization of CD3ε and IL-17A is visualized by orange immunofluorescence (bar = 20 μm). NS, not significant.

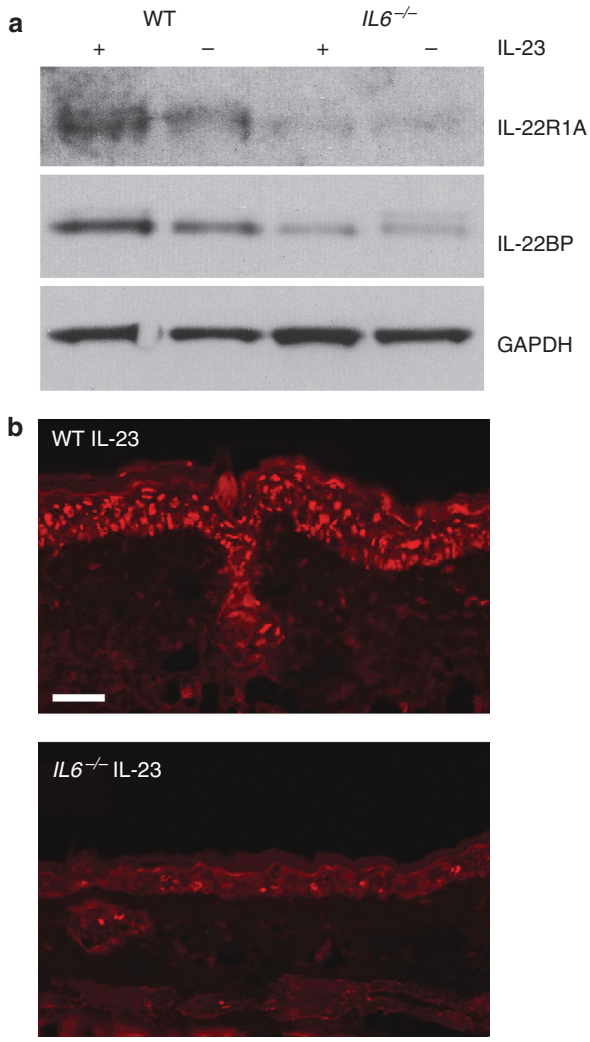


Figure 4. IL-22R1A expression is regulated by IL-6 expression. (**a**) Protein extracts were prepared from frozen ear biopsies of IL-23-injected WT and IL-6$^{-/-}$ mice and subjected to western blot. (**b**) Representative immunofluorescent stainings for IL-22R1A in IL-23-injected WT and IL-6$^{-/-}$ mouse ear tissue ($n=5$; bar = 20 μm).

hyperplasia via the IL-20Rβ following intradermal injections of IL-23 (Chan *et al.*, 2006; Zheng *et al.*, 2007). We show a marked upregulation of *Il-19* in lesional psoriatic skin and in mouse IL-23-induced dermatitis, supporting that signalling through the IL-20Rβ might mediate residual epidermal hyperplasia as previously suggested (Chan *et al.*, 2006).

Earlier studies have shown that IL-23 can activate the JAK/STAT signalling pathways (Parham *et al.*, 2002) in KCs (Tonel and Conrad, 2009) and in Th17 cells (Chen *et al.*, 2006). Correlating with these observations *Stat1*, *Stat3*, suppressor of cytokine signalling 3 (*Socs3*) and protein tyrosine phosphatase receptor type C (*Ptprc*) were upregulated at the mRNA level (Table 1) and a marked upregulation of both phosphorylated and non-phosphorylated STAT3 protein was seen in IL-23-injected mice (Supplementary Figure 1 online).

FACS data from our studies showed cellular infiltration of CD4$^+$ T cells, CD8$^+$ T cells, dendritic cells, macrophages, and neutrophils in IL-23-injected ears. Additionally, we observed a reduction in percentages of neutrophils in the draining lymph nodes following intradermal injections of IL-23, suggesting an active recruitment of neutrophils from the draining lymph nodes. In a previous study of IL-23-induced dermatitis, Chan *et al.* (2006) showed a similar infiltration of immune cell subsets in mouse back skin except for CD8$^+$ T cell infiltration, a feature also present in psoriasis. In another study by Hedrick *et al.* (2009), no differences were observed between IL-23-injected and control mice with respect to infiltration of macrophages, neutrophils or CD8$^+$ T cells. These differences could be due to varying time points of sampling. Our data is based on single-cell suspensions from ear skin prepared on day 10 at a time of T cell-dependent inflammation. Chan *et al.* (2006) performed immunohistochemistry staining on day 1 and day 4 possibly before onset of the T-cell-dependent phase of the response. Hedrick *et al.* (2009) did FACS analysis on day 15, a late time point where active inflammation may have started to decline.

In conclusion, we have shown that IL-23-induced dermal inflammation in mice is T-cell-dependent and, in particular, IL-6-dependent. Although IL-6-deficient mice respond to intradermal injections of IL-23 with enhanced IL-22 production compared with WT mice, this is insufficient for sustained dermal inflammation and acanthosis. Our findings strongly suggest that this is due to a failure to express significant levels of the specific IL-22 receptor (IL-22R1A) in the absence of IL-6.

MATERIALS AND METHODS

Human skin biopsies

Approval from the local Ethics Committee was obtained and the study was conducted according to the Declaration of Helsinki principles. Patients included had moderate-to-severe chronic plaque psoriasis and had not received any topical or systemic anti-psoriatic treatments for 2 and 4 weeks, respectively, before biopsy. After informed written consent was obtained, lesional and nonlesional keratome biopsies were taken under local anesthesia. The biopsies were snap-frozen in liquid nitrogen for RNA extraction and analysis.

Mice

Female C57BL/6JBomTac (6- to 8-week old; Taconic, Ry, Denmark) listed as WT in text; B6.129S2-*Il6tm1Kopf/J*, backcrossed for 11 generations to C57BL/6J mice (stock No. 00664, Jackson Laboratory, Bar Harbor, ME) listed as IL-6$^{-/-}$ mice in text were used for all experiments. The breading quality/health of the animals was guaranteed by the suppliers. Mice were kept at pathogen-free facilities at LEO Pharma, Denmark and were provided food and water *ad libitum*. All experiments were approved by the animal Ethics Committee.

Treatments

Mice were anesthetized with isoflurane and injected i.d. in the ear every other weekday for 10 days with 10 μl of mouse recombinant IL-23 (eBioscience, San Diego, CA) diluted 1:1 in 10% C57BL/6 mouse serum in phosphate-buffered saline (final concentration 50 μg ml^{-1}). Control mice were injected with 5% C57BL/6 mouse serum in phosphate-buffered saline. Ear thickness was measured using an engineer's calliper (Mitutoyo, Aurora, IL) every weekday before injections. The rationale for injections into mouse ear tissue

was based on the feasibility of measuring ear swelling during the course of the experiment in addition to epidermal thickness as final end point.

Mice received drug treatment administered p.o. either vehicle 1% methylcellulose (LEO Pharma, Ballerup, Denmark) twice daily at 0.1 ml per 10 g mouse or CsA (Alexis Biochemicals, Switzerland) twice daily at 20 mg kg^{-1} mouse or Dexamethasone (Dexadresone Intervet, Boxmeer, The Netherlands) once daily at 2 mg kg^{-1} mouse. During weekends mice were treated once daily regardless of drug treatment. At the end of experiments, mice were killed by isoflurane anesthetization followed by cervical dislocation.

Tissue sampling and measurement of epidermal thickness

Biopsies (8-mm thick) from the ears covering most of the injected ear surface were halved and placed incision-side down in Tissue-Tek O.C.T. Compound (Sakura Finetek, Alphen aan den Rijn, The Netherlands) and snap-frozen in liquid nitrogen. Biopsies were sectioned on a cryostat microtome (MICROM HM 560, Thermo Fischer Scientific, Walldorf, Germany). Sections were stained with Masson's Trichrome and mean epidermal thickness determined using Visiopharm software (Visiopharm, Hoersholm, Denmark).

Immunohistochemistry

For staining of proliferative cells, polyclonal rabbit anti-mouse Ki67, clone SP6 (Histolab, Gothenburg, Sweden, 1:100) was used. The cytokine IL-17A was stained by rabbit anti-human IL-17A (Santa Cruz Biotechnology, Santa Cruz, CA, 1:100) and CD3-positive cells by hamster polyclonal anti-mouse CD3e (Invitrogen, Carlsbad, CA, 1:400) and rat anti-mouse IL-22R1a (clone 496514, R&D Systems, Abingdon, UK, 1:100). Detailed descriptions of the immunohistochemical procedures can be found in Supplementary methods online.

RNA extraction and qPCR analysis

Biopsies from pooled lesional or matched nonlesional psoriatic skin ($n=5$) were crushed under liquid nitrogen. Ear biopsies of mice from each treatment group ($n=5$) were pooled and disintegrated in a TissueLyser (Qiagen, Haan, Germany). RNA was extracted by use of the RNeasy Lipid Tissue Mini Kit (Qiagen, Germantown, MD) and reverse-transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). The samples were amplified by real-time quantitative PCR (qPCR) using Applied Biosystems validated gene expression assays and PRISM 7900HT sequence detection system (SDS 2.3). Fold changes of mRNA expression were calculated by the comparative Ct method and normalized to GAPDH using the RealTime StatMiner software (Integromics, Granada, Spain).

Western blot

IL-22 Receptor Chains were detected by western blot using the specific primary antibodies IL-22R1a (rat anti-mouse IL-22R1a, R&D systems, Clone #496504-22) and IL-22BP (goat anti-mouse IL-22BP, Santa Cruz Biotechnology, Sc-67638). GAPDH detection was used for loading control. Further details are described in Supplementary Methods online.

Cytokine determination

Cytokines from homogenized ear biopsies were measured by Mouse Pro-inflammatory 7-Plex Ultra-Sensitive and Mouse TH1/TH2 9-Plex Ultra Sensitive Kits (Meso Scale Discovery, Gaithersburg, MD) on a meso scale discovery platform. For analysis of Th17 cytokines, Mouse IL-17A Quantikine Immunoassay and Mouse/Rat IL-22 Quantikine Immunoassay (R&D Systems) were used. Total amounts of protein in each sample were quantified by the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Further details are described in Supplementary methods online.

CONFLICT OF INTEREST

The authors of this publication were employees of the company funding the research.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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