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Pathogenic function of IL-1β in psoriasiform skin lesions of flaky skin (*fsn/fsn*) mice

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SUMMARY

IL-1 acts on many cells as an inflammatory mediator. Its two forms, IL-1α and IL-1β, are regulated differentially within hyperproliferative inflammatory skin conditions, such as psoriasis. While IL-1α is down-regulated within psoriatic lesions, the levels of IL-1β are increased. However, some investigators have described an inactive form of IL-1β in psoriasis, while others have detected increased IL-1β activity within these lesions. Thus, its *in vivo* role remains unclear. We have assessed expression and function of IL-1β within psoriasiform skin lesions of the spontaneous mouse mutation flaky skin (*fsn/fsn*). It was found that IL-1β was increased by 357% within psoriasiform lesions of *fsn/fsn* mice compared with their wild-type or heterozygous (+/?) littermates ($P < 0{\cdot}00001$). When the IL-1β function was inhibited by i.p. injection with a neutralizing MoAb, no effects were seen in +/? mice. In contrast, psoriasiform features in *fsn/fsn* mice were alleviated dramatically, as demonstrated by a 40% decrease of the epidermal thickness and a diminished number of intra-epidermal microabscesses. In addition, infiltrating epidermal CD4$^+$ and CD8$^+$ T cells were decreased by 68% and 81%, respectively ($P < 0{\cdot}05$), and epidermal Langerhans cells also were reduced by 36% ($P < 0{\cdot}005$). In contrast, mast cells were not affected, suggesting differential responses of various cutaneous cell types. Our results demonstrate an important *in vivo* role of IL-1β for the generation of hyperproliferative inflammatory skin lesions in the *fsn/fsn* model.

Keywords cytokines IL-1β psoriasis flaky skin inflammation

INTRODUCTION

Cytokines play key roles in the pathogenesis of inflammatory disorders, such as psoriasis, a common immunologically based human skin disease affecting 1–3% of the population [1]. *In vitro* studies have revealed cytokine effects which may explain the complex tissue alterations seen in psoriasis and other hyperproliferative inflammatory conditions, leading to the well-founded hypothesis of a cytokine network underlying the pathogenesis of the intertwined histopathological alterations in psoriasis [2]. The two forms of IL-1, IL-1α and IL-1β, are regulated differentially within psoriatic lesions. In particular, increased levels of IL-1β have been detected within psoriatic lesions compared with uninvolved skin, while IL-1α is down-regulated [3–7]. However, the *in vivo* roles of IL-1α and IL-1β in hyperproliferative inflammatory lesions are not completely clear. Although IL-1α is expressed at markedly decreased levels in psoriatic lesions compared with uninvolved skin, the still detectable biological activity of IL-1 was entirely attributable to IL-1α, suggesting that IL-1β was present in a non-functional form [8–10]. These studies were performed using epidermis-derived IL-1β from keratotome shave biopsies [8]. In another study, prominent expression of IL-1β has been demonstrated by *in situ* hybridization focally within the epidermis, but also within the dermis [11], and two studies demonstrated the presence of biologically active IL-1β in psoriatic scales [12,13]. In addition, the number of activated mast cells is increased in the dermis of psoriatic lesions [14–16], and mast cell-produced chymase can rapidly activate IL-1β [17], although the relevance of this mechanism in inflammatory skin disorders is unclear. Thus, it is conceivable that IL-1β may play a role, at least in certain stages of the pathogenesis of hyperproliferative inflammatory skin disorders. However, while some transgenic mice over-expressing IL-1α in the basal epidermal layer develop spontaneous inflammatory skin lesions [18], no such observations have been reported for IL-1β.

To assess further a potential *in vivo* role of IL-1β in the generation of hyperproliferative inflammatory skin lesions, we have studied its expression and function in the skin of flaky skin (*fsn/fsn*) mice. *Fsn* is a spontaneous autosomal recessive mouse mutation mapped to chromosome 17 and characterized by

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multiorgan abnormalities including prominent erythrosquamous skin lesions [19]. While the flaky skin mutation is not an animal equivalent of human psoriasis, the cutaneous disorder is characterized by epidermal hyperplasia with ortho-hyperkeratosis, focal parakeratosis, angiogenesis and dilation of blood vessels, and a mixed inflammatory infiltrate including epidermal microabscesses and an increased number of dermal mast cells [20,21]. Thus, although the *fsn*-mutation does not share the proposed immunopathogenesis with psoriasis, it appears to be a useful model for studying local events resulting in hyperproliferative inflammatory alterations of the skin [20,22,23].

Here, we quantitatively assessed expression of IL-1β at the protein level within cutaneous lesions of *fsn/fsn* mice compared with normal littermates, and studied the effect of *in vivo* blockade of IL-1β on the psoriasiform phenotype. We present data showing that, similar to psoriasis, IL-1β is markedly elevated within the psoriasiform skin lesions of *fsn/fsn* mice. In addition, we demonstrate that *in vivo* neutralization of this cytokine can alleviate the hyperproliferative inflammatory lesions of *fsn/fsn* mice.

MATERIALS AND METHODS

Animals

Breeding pairs of CBy.A fsn/J mice (The Jackson Laboratory, Bar Harbor, ME) were maintained in a specific pathogen-free environment in a barrier facility. They were kept at a 12-h daily lighting period, 50–70% relative humidity, and a temperature of 19–23°C in type-3 cages. Mice received autoclaved food (no. 1314; Altromin, Lage, Germany) and water (adjusted with HCl to pH 2·5–3·0 to prevent growth of microorganisms). As the genetic defect resulting in the flaky skin phenotype is unknown and as homozygous mutant mice are not fertile [20], the offspring of CBY$^{FSN/fsn}$ mice was used for all experiments. In the CBy.A background, erythrosquamous skin lesions were readily seen at the age of 5–6 weeks, allowing the separation of *fsn/fsn* mice from their wild-type or heterozygous littermates (hereafter +/?). For cytokine detection by ELISA and antibody treatment studies, mice were used between 12 and 16 weeks of age (littermates in most cases), after it had been established that the phenotype remained stable within this time frame.

Cytokine detection by ELISA

Homogenates were prepared from snap-frozen whole dorsal skin biopsies at a fixed tissue:buffer ratio (6-mm punch biopsy/0·5 ml buffer) using a dismembrator (Braun, Melsungen, Germany) for 1 min at 2600 beats/min, followed by resuspension in 1% SDS (in buffer containing 10 mM Tris, 1 mM EDTA; all from Merck, Darmstadt, Germany) and another round of mechanical homogenization. Thereafter, samples were homogenized for 5 min in an ultrasound bath (Elma, Darmstadt, Germany), and spun at 14 000 ***g*** for 5 min. The supernatant was collected, total protein was quantified fluorometrically using a Bradford assay, and quantification of IL-1β and, for control, IL-10 was performed by ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Briefly, 50 μl of the sample solution, the standard solution, or the control solution were mixed with 50 μl of the diluent RD1W, transferred into a microtitre plate, and incubated at room temperature for 2 h. Each well was then washed × 5 with buffer (PBS containing one tablet/50 ml of Complete® protease inhibitor; Boehringer, Mannheim, Germany), incubated with 100 μl cytokine-conjugate for 2 h at room temperature, and washed × 5 again. Thereafter, each well was incubated with 100 μl of the substrate solution, and the reaction was stopped after 30 min. Bound cytokines were quantified using a EAR 340 ATC spectrophotometer (SLT Labinstruments, Hamburg, Germany) at 450 and 570 λ.

In vivo *antibody treatment studies*

Mice were injected intraperitoneally with 200 μg of the neutralizing 30311.11 MoAb (rat IgG1 anti-mouse IL-1β; R&D Systems, Wiesbaden, Germany) in 200 μl PBS as described recently for other MoAbs [24]. Mice received four injections at 2-day intervals. Control groups received 200 μg R59-40 MoAb (rat IgG1; PharMingen, San Diego, CA) in 200 μl PBS. Five +/? and five *fsn/fsn* mice were included in each treatment group. Penetration of MoAb into the skin was confirmed by direct immunostaining of cryostat-cut sections using a biotinylated mouse-adsorbed anti-rat IgG (BA-4001 from Vector (Burlingame, CA)).

Cell culture and antibody purification

Hybridoma cell lines were maintained in RPMI 1640 supplemented with 10% immunoglobulin-depleted fetal calf serum (FCS), 10^{-5} M β-mercaptoethanol, 1% non-essential amino acids, 1% L-glutamine, 1% penicillin/streptomycin/amphotericin, 15 mM HEPES, and cultured at 37°C and 5% CO_2. MoAbs were purified from culture supernatant by affinity chromatography using a Protein G-Sepharose column (Pharmacia Biotech, Erlangen, Germany). Bound MoAb was eluted using 0·5 M acetic acid, neutralized with 1:20 volume of 1 M Tris pH 8·0, and dialysed against PBS overnight. Concentration of MoAb was determined fluorometrically (Bradford assay), and for *in vivo* studies, MoAbs were diluted at 1 mg/ml in PBS. Immunostaining of cryostat-cut sections of mouse skin confirmed the specificity of the purified MoAbs. The protein G-Sepharose columns were regenerated by elution with 1 M acetic acid pH 2·5.

Monoclonal antibodies

Hybridomas producing the following MoAbs were cultured: M1/70 (anti-CD11b (α_M-integrin), rat IgG2b; ATCC, Rockville, MD), 53-6.7 (CD8α, rat IgG2a; ATCC), YN1/1.7.4 (anti-CD54 (ICAM-1), rat IgG2a; ATCC), N22 (anti-MHC class II, hamster IgG; ATCC). The following MoAbs were purchased: R59-40 (rat IgG1-control; PharMingen), R35-95 (rat IgG2a-control; PharMingen), R35-38 (rat IgG2b-control; PharMingen), UC8-4B3 (hamster IgG-control; PharMingen), 500A2 (anti-CD3ε, hamster IgG; PharMingen), and RM4-5 (CD4, rat IgG2a; PharMingen). Biotinylated goat anti-hamster serum and mouse adsorbed rabbit anti-rat serum were purchased from Vector Labs Inc.

Histochemical and immunohistochemical analysis

Haematoxylin and eosin (H–E) or Giemsa staining of 3-μm sections of paraffin-embedded tissue were performed according to standard protocols. Chloroacetate-esterase staining was performed as described previously [25].

For immunohistochemistry, 5-μm cryostat-cut sections were stained by the immunoperoxidase method using 10 μg/ml of the primary MoAb and 3-amino-9-ethylcarbazole as chromogen according to the manufacturer's instructions (ABC; Vector). Slides were counterstained with haematoxylin and $LiCO_3$.

Morphometric and statistical analysis

Stained sections from each mouse were scanned using the Nikon

Coolscan II® software. Epidermal thickness was assessed at 10 different points in each H–E-stained section, and the average thickness was calculated. Infiltrating leucocytes were evaluated as cells/mm dermo–epidermal junction. When dermal cells were counted, the full depth of the biopsy was evaluated. Data are represented as mean ± s.d. ($n = 5$). Statistical significance was assessed by the paired two-tailed Student's *t*-test, and $P < 0{\cdot}05$ was considered significant.

RESULTS

IL-1β is over-expressed in the skin of fsn/fsn mice

In the offspring of heterozygous intercrosses, erythrosquamous skin lesions first appeared at 5–6 weeks of age. No further increase in clinical severity was apparent after 11 weeks of age. As described previously, there was a 7·5-fold thickening of the viable epidermal layers (acanthosis) in homozygous mutant mice compared with +/? littermates [20,24]. This was associated with profound hyperkeratosis, dilation and increase of dermal blood vessels, and a mixed inflammatory infiltrate composed of lymphocytes, macrophages, mast cells, and neutrophils, similar to other rodent models of hyperproliferative inflammatory skin disorders [26–28].

Dorsal skin from *fsn/fsn* and +/? mice ($n = 6$ for each genotype) was harvested to assess IL-1β within the skin. As detected by ELISA, IL-1β expression was dramatically increased by 357% within the hyperproliferative inflammatory lesions of *fsn/fsn* mice compared with their +/? counterparts (Fig. 1a). In contrast, the control cytokine, IL-10, was expressed at similar levels in *fsn/fsn* and +/? mice (0·16 pg/ml in *fsn/fsn* skin (s.d. = 0·009) *versus* 0·14 pg/ml in +/? skin (s.d. = 0·026); $P = 0{\cdot}075$; data not shown).

Immunohistochemical analyses revealed that IL-1β reactivity was almost absent in the skin of +/? mice ($n = 5$), while IL-1β expression was readily detected diffusely in the dermis as well as focally within the epidermis of *fsn/fsn* mice ($n = 5$, Fig. 1b). Thus, expression within both dermis and epidermis appeared to contribute to the elevated levels of IL-1β in the hyperproliferative inflammatory skin lesions of *fsn/fsn* mice detected by ELISA.

Psoriasiform features in fsn/fsn mice are alleviated by neutralizing IL-1β in vivo

As cutaneous expression of IL-1β in *fsn/fsn* mice was markedly higher than that in +/? mice, we sought to neutralize IL-1β to assess directly its role in the pathogenesis and maintenance of the murine hyperproliferative inflammatory lesions *in vivo*. +/? and *fsn/fsn* mice were injected intraperitoneally four times at 2-day intervals with 200 μg of the neutralizing anti-IL-1β MoAb 30311.11. Control mice received an isotype-matched antibody. Penetration of the MoAbs into the skin of the recipient mice was confirmed by immunohistochemistry using a goat anti-rat antibody to detect the injected MoAbs within the skin (not shown).

When the skin of the treated mice was examined histopathologically, no apparent effect of the anti-IL-1β treatment was seen in +/? mice (Fig. 2 and Table 1). In contrast, the epidermal thickness was dramatically reduced by 40·3% in anti-IL-1β-treated *fsn/fsn* mice compared with control *fsn/fsn* mice (0·362 mm (s.d. = 0·05) *versus* 0·606 mm (s.d. = 0·05), $P < 0{\cdot}001$, Fig. 2 and Table 1). This was accompanied by a diminished inflammatory infiltrate and a reduced number of dermal blood vessels (Fig. 2). In addition, the number of epidermal microabscesses within the skin of anti-IL-1β-treated *fsn/fsn* mice appeared to be reduced compared with isotype-treated mice (5·6 abscesses/mm (s.d. = 1·1) *versus* 3·1 abscesses/mm (s.d. = 1·3)), although this difference did not achieve statistical significance.

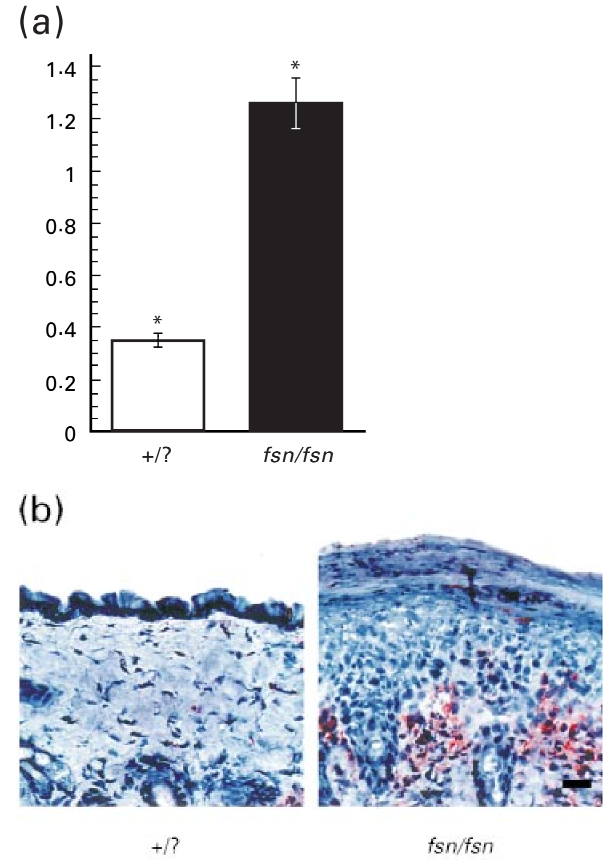

Fig. 1. IL-1β expression is elevated within psoriasiform lesions of *fsn/fsn* mice compared with +/? littermates. (a) IL-1β was quantitatively assessed by ELISA using equal amounts of total protein from whole-skin extracts of +/? mice (□) and *fsn/fsn* mice (■) as outlined in Materials and Methods. The columns represent average amounts of IL-1β from six animals in each group (± s.d.). *$P < 0{\cdot}00001$. (b) IL-1β was detected by immunohistochemistry in 5-μm sections of dorsal skin from a +/? mouse (left panel) and a *fsn/fsn* mouse (right panel). Scale bar = 20 μm. The panels shown are representative of similar results seen with five animals in each group.

Neutralizing IL-1β in vivo differentially affects cutaneous cell types in fsn/fsn mice

To examine the effect of IL-1β in more detail, the distribution and number of infiltrating T lymphocytes, granulocytes, macrophages, epidermal dendritic cells (Langerhans cells), and dermal mast cells were assessed in +/? and *fsn/fsn* mice treated with an isotype-matched control MoAb or the anti-IL-1β MoAb (Fig. 3, data summarized in Table 1). Based upon immunohistochemical analyses of frozen sections, no infiltrating CD3$^+$ T lymphocytes were detected in the skin of control +/? mice, and treatment with the anti-IL-1β MoAb did not cause any alterations. In contrast, there were abundant CD3$^+$ T cells within the hyperproliferative inflammatory lesions of *fsn/fsn* mice, and treatment with the neutralizing anti-IL-1β MoAb resulted in dramatic reduction of the infiltrating T cells. In all animals, epidermal T lymphocytes

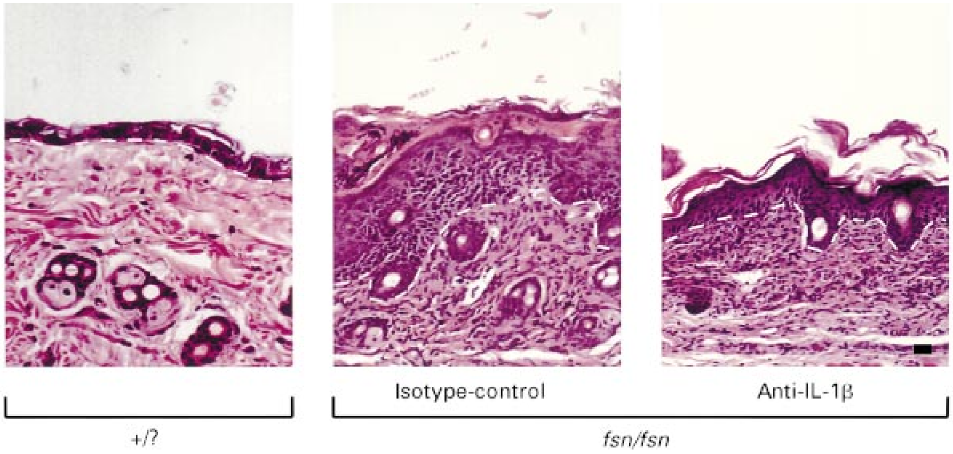

Fig. 2. *In vivo* neutralization of IL-1β alleviates psoriasiform skin lesions in *fsn/fsn* mice. +/? mice (left panel) and *fsn/fsn* mice (middle and right panels) were injected intraperitoneally with an isotype-matched control MoAb (middle panel) or the anti-IL-1β MoAb 30311.11 (left and right panels) as described in Materials and Methods. Dorsal skin was harvested 9 days after initiation of the treatment, and 3 μm paraffin-embedded sections were stained by haematoxylin and eosin. The dashed lines indicate the location of the dermo–epidermal junction. Scale bar = 20 μm. The panels shown are representative of five mice in each treatment group.

were preferentially located within the basal and one or two suprabasal layers of the epidermis. Infiltrating T cells within the psoriasiform skin lesions of *fsn/fsn* mice were predominantly of the CD4^{+} phenotype, while only few CD8^{+} T cells were seen. When IL-1β was neutralized *in vivo*, the numbers of epidermal CD4^{+} and CD8^{+} T cells were reduced significantly. In addition, CD11b^{+} epidermal cells (mostly neutrophils) and epidermal MHC class II^{+} dendritic cells (Langerhans cells) were markedly diminished by neutralizing IL-1β. In contrast to the above cell types and as detected by both Giemsa (Fig. 3b) and chloroacetate esterase (data not shown) staining, the number of dermal mast cells in the skin of *fsn/fsn* mice was not affected significantly by treatment with the anti-IL-1β MoAb (Fig. 3b and Table 1). These results indicate that cutaneous cell types in *fsn/fsn* mice responded differentially to neutralization of IL-1β.

DISCUSSION

Within the erythrosquamous skin lesions of *fsn/fsn* mice, hyperproliferative inflammatory changes were associated with a 3·5-fold increased expression of IL-1β. Indeed, the higher cellularity of *fsn/fsn* skin may have resulted in extra dilution of proteins during sample preparation for ELISA, and the IL-1β content in *fsn/fsn* skin samples may have been even higher than detected here. These features are shared between this disorder and psoriasis [3–6,8]. As the role of IL-1β in psoriasis has not yet been completely clarified, we have used the *fsn/fsn* model to directly demonstrate a pathogenic function of IL-1β in hyperproliferative inflammatory skin alterations *in vivo*. Antibody-mediated neutralization of IL-1β resulted in significant alleviation of psoriasiform skin lesions in *fsn/fsn* mice. These results add to our understanding of the pathogenesis of the *fsn/fsn* phenotype [19,20], which is not completely understood.

An increased number of MHC class II^{+} epidermal dendritic cells in the skin of *fsn/fsn* mice, which has been described previously [29] and confirmed here, suggested immunological abnormalities in this model. As demonstrated in this study, abundant T lymphocytes are also present within the skin of *fsn/fsn*

Table 1. Effect of *in vivo* neutralization of IL-1β on hyperproliferative inflammatory skin alterations in flaky skin (*fsn/fsn*) and wild-type (+/?) mice ($n = 5$ in each group)

Genotype Treatment	*fsn/fsn* Isotype control	*fsn/fsn* Anti-IL-1β	+/? Isotype control	+/? Anti-IL-1β
Epidermal thickness (mm)	0·606 ± 0·053	0·362 ± 0·047**	0·077 ± 0·003	0·078 ± 0·007
Epidermal CD3^{+} T cells/mm	74·8 ± 12·83	24·6 ± 8·34*	7·3 ± 2·1	7·9 ± 1·5
Epidermal CD4^{+} T cells/mm	66·4 ± 18·23	21·3 ± 7·26*	0	0
Epidermal CD8^{+} T cells/mm	4·87 ± 1·41	0·93 ± 0·12*	0	0
Epidermal MHC II^{+} dendritic cells/mm	47·87 ± 3·93	30·87 ± 3·56**	23·9 ± 1·1	23·2 ± 1·3
Epidermal CD11b^{+} cells/mm	22·2 ± 2·49	12·0 ± 1·60*	0	0
Dermal mast cells/mm	120·6 ± 26·43	101·66 ± 9·18	9·5 ± 0·98	9·9 ± 1·67

Adult mice were injected intraperitoneally four times with 200 μg of the neutralizing IL-1β-specific MoAb 30311.11 or an isotype-matched control MoAb at 2-day intervals. Dorsal skin was harvested 9 days after initiation of the treatment, and analysed by H–E histology and immunohistochemistry.
$*P < 0·05$; $**P < 0·005$ comparing mice treated with isotype-matched MoAbs with mice treated with anti-IL-1β MoAbs.

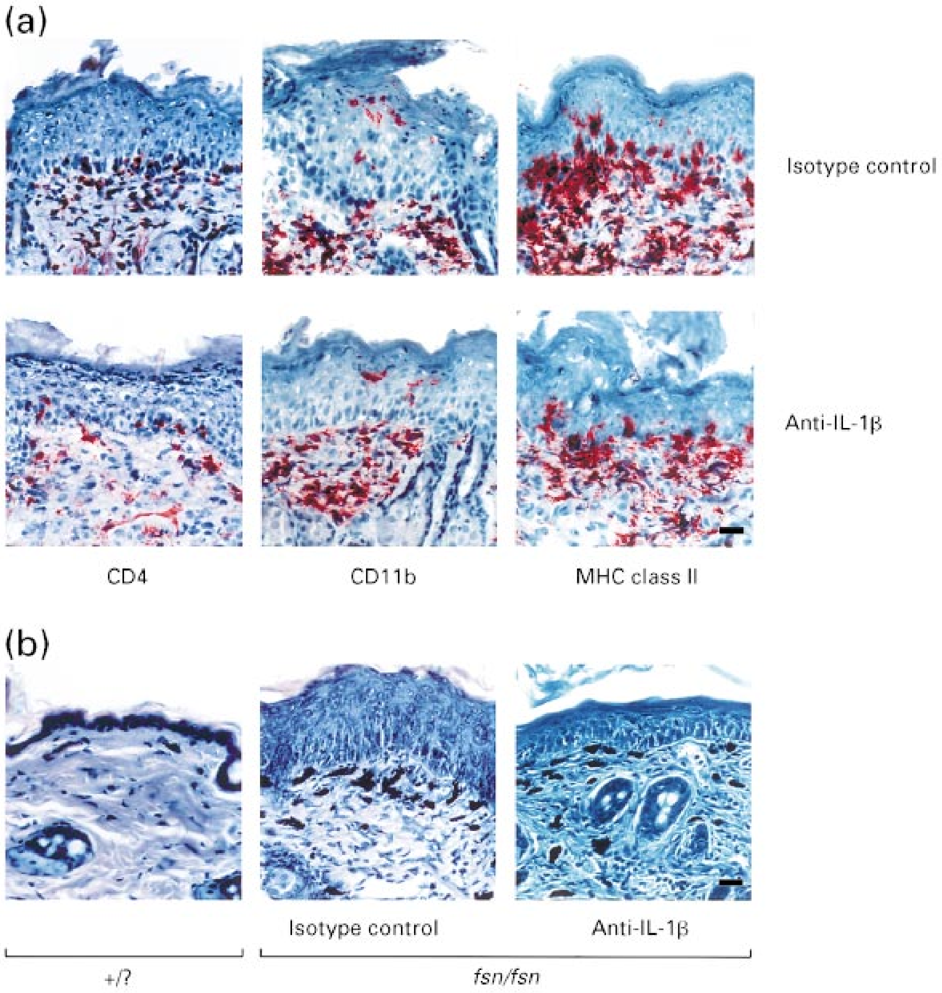

Fig. 3. *In vivo* neutralization of IL-1β differentially affects cutaneous cell types in *fsn/fsn* mice. (a) Homozygous mutant *fsn/fsn* mice ($n = 5$ in each group) were treated by i.p. injections with an isotype-matched control MoAb (upper row) or the IL-1β-neutralizing MoAb (lower row). Leucocyte antigens were detected by immunohistochemistry as indicated in 5-μm cryostat-cut sections of dorsal skin. The panels shown are representative of five mice in each treatment group. Scale bar = 20 μm. (b) +/? mice ($n = 5$) and *fsn/fsn* mice ($n = 5$) were injected as outlined in (a) paraffin-embedded sections (3 μm) of dorsal skin from anti-IL-1β-treated +/? mice (left), isotype-treated *fsn/fsn* mice (middle panel) and anti-IL-1β-treated *fsn/fsn* mice (right) were Giemsa-stained. Cutaneous mast cells are visualized as dark purple cells. Scale bar = 20 μm.

mice. However, cutaneous lesions do not appear to be induced by T cells, as they develop in *scid/scid fsn/fsn* double mutant mice [20] which lack mature B and T cells [30]. In addition, cyclosporin A was not effective when used for treating *fsn/fsn* lesions [20]. Neutrophilic granulocytes, however, appear to play an important role in the pathogenesis of the *fsn/fsn* phenotype [24]. Over-expression of epidermal growth factor-receptor (EGF-R) in *fsn/fsn* skin [31] suggests that this disorder may also entail intrinsic epidermal abnormalities. Thus, the pathogenesis of the *fsn/fsn* phenotype is still obscure. Our results now demonstrate that IL-1β is also an important pathogenic factor for the generation of hyperproliferative inflammatory skin lesions in *fsn/fsn* mice.

When different cell types were analysed in the skin of *fsn/fsn* mice after neutralization of IL-1β, differential responses became apparent inasmuch as epidermal acanthosis and hyperproliferation as well as cutaneous T cell and neutrophil infiltration and MHC class II^{+} epidermal dendritic cells were markedly diminished, while mast cells were not affected significantly. Although it has not formally been ruled out that treatment for a longer period of time would have elicited a mast cell response as well, the mast cell response would at least be slower than that of the other cell types. Thus, it appears that cutaneous cell types responded differentially to IL-1β, either directly or indirectly via secondary cytokines.

In addition, our results may carry implications for the pathogenesis of human hyperproliferative inflammatory skin disorders, such as psoriasis. As skin lesions of *fsn/fsn* mice resemble a number of histopathological aspects seen in psoriasis, they appear to be a useful model for studying local events leading to the development of hyperproliferative inflammatory skin changes [23]. Thus, it is conceivable that IL-1β also plays a role in the pathogenesis of psoriasis. The detection of an inactive form within psoriatic epidermis [8,9] argues against this possibility, and it is possible that IL-1β is not critically involved in the pathogenesis of psoriasis. However, as these results were obtained with epidermal IL-1β, and IL-1β mRNA has also been detected within the psoriatic dermis [11], it is possible that the dermal IL-1β contributes to the generation of the psoriatic phenotype. In addition, biologically active forms of IL-1β have been detected in psoriatic epidermis [12,13], in one study after separation from IL-1 receptor antagonist [13]. In any case, our results demonstrate that IL-1β can be critically involved in the generation of hyperproliferative inflammatory skin alterations, at least in the *fsn/fsn* model.

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REFERENCES

1 Christophers E, Sterry W. Psoriasis. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Dermatology in general medicine. New York: McGraw-Hill Inc., 1993:489–514.

2 Nickoloff BJ. The cytokine network of psoriasis. Arch Dermatol 1991; **127**:871–84.

3 Kim N-I, Cooper KD, Fisher GJ *et al.* Psoriatic skin reveals the in vivo presence of an epidermal IL-1 inhibitor. Arch Dermatol Res 1992; **284**:71–76.

4 Prens EP, Benne K, van Damme J *et al.* Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 1990; **95**:121S–124S.

5 Uyemura K, Yamamura M, Fivenson DF *et al.* The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 1993; **101**:701–5.

6 Yoshinaga Y, Hagaki M, Terajima S *et al.* Detection of inflammatory cytokines in psoriatic skin. Arch Dermatol Res 1995; **287**:158–64.

7 Debets R, Hegmans JPJJ, Croughs P *et al.* The IL-1 system in psoriatic skin. IL-1 antagonist sphere of influence in lesional psoriatic epidermis. J Immunol 1997; **158**:2955–63.

8 Cooper KD, Hammerberg C, Baadsgaard O *et al.* IL-1 activity is reduced in psoriatic skin: decreased IL-1α and increased non-functional IL-1β. J Immunol 1990; **144**:4593–603.

9 Cooper KD, Hammerberg C, Baadsgaard O *et al.* Interleukin-1 in human skin: dysregulation in psoriasis. J Invest Dermatol 1990; **95**:24S–26S.

10 Ohta Y, Katayama I, Funato T *et al.* In situ expression of messenger RNA of interleukin-1 and interleukin-6 in psoriasis: interleukin-6 involved in formation of psoriatic lesions. Arch Dermatol Res 1991; **283**:351–6.

11 Schmid P, Cox D, McMaster GK *et al.* In situ hybridization analysis of cytokine, proto-oncogene and tumor suppressor gene expression in psoriasis. Arch Dermatol Res 1993; **285**:334–40.

12 Debets R, Hegmans TPJJ, Troost RJJ *et al.* Enhanced production of biologically active interleukin-1α and interleukin-1β by psoriatic epidermal cells ex vivo: evidence of increased cytosolic interleukin-1β levels and facilitated interleukin-1 release. Eur J Immunol 1995; **25**:1624–30.

13 Nylander Lundquist E, Egelrud T. Biologically active, alternatively processed interleukin-1β in psoriatic scales. Eur J Immunol 1997; **27**:2165–71.

14 Brody I. Mast cell degranulation in the evolution of acute eruptive guttate psoriasis vulgaris. J Invest Dermatol 1984; **82**:460–4.

15 Schubert C, Christophers E. Mast cells and macrophages in early relapsing psoriasis. Arch Dermatol Res 1985; **277**:352–8.

16 Brody I. Mast cell alterations in chronic psoriasis vulgaris; response to low-strength anthralin treatment. A transmission electron microscopic study. Uppsala J Med Sci 1986; **91**:1–16.

17 Mizutani H, Schecht N, Lazarus G *et al.* Rapid and specific conversion of precursor interleukin-1β (IL-1β) to an active IL-1 species by human mast cell chymase. J Exp Med 1991; **174**:821–5.

18 Groves RW, Mizutani H, Kieffer JD *et al.* Inflammatory skin disease in transgenic mice that express high levels of interleukin 1α in basal epidermis. Proc Natl Acad Sci USA 1995; **92**:11874–8.

19 Beamer WG, Pelsue SC, Shultz LD *et al.* The flaky skin (fsn) mutation in mice: map location and description of the anemia. Blood 1995; **86**:3220–6.

20 Sundberg JP, Boggess D, Shultz LD *et al.* The flaky skin (fsn) mutation, chromosme? In: Sundberg JD, ed. Handbook of mouse mutations with skin and hair abnormalities. Boca Raton: CRC Press, 1994: 253–68.

21 Pelsue SC, Schweitzer PA, Schweitzer IB *et al.* Lymphadenopathy, elevated serum IgE levels, autoimmunity, and mast cell accumulation in flaky skin mutant mice. Eur J Immunol 1998; **28**:1379–88.

22 Sundberg JG, Beamer WG, Shultz LD *et al.* Inherited mouse mutations as models of human adnexal, cornification, and papulosquamous dermatoses. J Invest Dermatol 1990; **95**:62S–63S.

23 Schön MP. Animal models of psoriasis—what can we learn from them? J Invest Dermatol 1999; **112**:405–10.

24 Schön M, Denzer D, Kubitza RC *et al.* Critical role of neutrophils for the generation of psoriasis skin lesions in flaky skin mice. J Invest Dermatol 2000; **114**:976–83.

25 Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 1971; **55**:283–90.

26 Carroll JM, Romero MR, Watt FM. Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. Cell 1995; **83**:957–68.

27 Hammer RE, Maika SD, Richardson JA *et al.* Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human b2m: an animal model of HLA-B27-associated human disorders. Cell 1990; **63**:1099–112.

28 Schön MP, Detmar M, Parker CM. Murine psoriasis-like disorder induced by naive CD4+ T-cells. Nature Med 1997; **3**:183–8.

29 Sundberg JG, Boggess D, Sundberg BA *et al.* Epidermal dendritic cell populations in the flaky skin mouse mutant. Immunol Invest 1993; **22**:389–401.

30 Bosma MJ, Carroll AM. The SCID mouse mutant: definition, characterization, and potential uses. Ann Rev Immunol 1991; **9**:323–50.

31 Nanney LB, Sundberg JP, King LE. Increased epidermal growth factor receptor in fsn.fsn mice. J Invest Dermatol 1996; **106**:1169–74.