IL-1RL2 and Its Ligands Contribute to the Cytokine Network in Psoriasis

Jennifer E. Towne and John E. Sims

Hal Blumberg, Huyen Dinh, Charles Dean, Jr., Esther S. Trueblood, Keith Bailey, Donna Shows, Narasimharao Bhagavathula, Muhammad Nadeem Aslam, James Varani, Jennifer E. Towne and John E. Sims

*J Immunol* 2010; 185:4354-4362; Prepublished online 10 September 2010;
doi: 10.4049/jimmunol.1000313
http://www.jimmunol.org/content/185/7/4354

Supplementary Material http://www.jimmunol.org/content/suppl/2010/09/08/jimmunol.1000313.DC1

References This article cites 63 articles, 22 of which you can access for free at: http://www.jimmunol.org/content/185/7/4354.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2010 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Psoriasis is a chronic skin disease characterized by epidermal hyperproliferation and altered differentiation, an inflammatory cell infiltrate in the epidermis and dermis, and an increase in and dilation of superficial dermal blood vessels (1). The disease involves patches of lesional skin separated from each other by normal-appearing skin. T cells have been implicated as key players in psoriasis by the finding that agents affecting T cell survival, activation, or function demonstrate clinical efficacy (1, 2).

Psoriasis has been difficult to model in the mouse because of profound differences in the structure and development of mouse and human skin (3, 4). The most widely accepted models involve transplantation of human skin from psoriasis patients onto immunodeficient mice (5, 6). Transplanted lesional skin retains its psoriatic characteristics, but it can be normalized by agents that are clinically effective in humans (4). Transplanted nonlesional skin from psoriasis patients can be converted to the psoriatic phenotype by the patient’s own activated T cells, but skin from normal individuals treated in the same way cannot, suggesting an abnormality in the skin in addition to the altered function of T cells from patients (5–7). Genetic and ex vivo studies also provide support for a role of keratinocytes in disease (8–13).

Psoriasis appears to involve a cytokine network centered around IL-17, IL-22, IL-23, and TNF, all of which are elevated in lesional skin (14–17). IL-22 (along with its related cytokines IL-19, IL-20, and IL-24) was shown to cause epidermal hyperplasia, primarily by downregulation of genes involved in terminal keratinocyte differentiation (18, 19). IL-17 acts on keratinocytes to induce chemokines that lead to neutrophil, inflammatory dendritic cell (TNF/INOS-producing dendritic cells, or TIP-DC) (20), and Th17 cell influx into the skin (21). IL-17 and IL-22 induce keratinocytes to produce antimicrobial peptides, such as S100A8, S100A9, β-defensin 2, and LL-37, known to be elevated in lesional skin (22–24). These protect against infection and act as endogenous ligands for TLRs, such as TLR4 and TLR9 expressed on keratinocytes and dendritic cells (DCs) (25, 26). Human β-defensin 2 is also chemotactic for CCR6+ cells (27), which include neutrophils, DCs, and Th17 cells, and for CCR2+ cells (28), which include TIP-DCs. IL-23 enhances the production of IL-17 and IL-22 from Th17 and other cells (29, 30). TIP-DCs make inducible NO synthase (leading to NO, which can dilate dermal blood vessels) and secrete TNF, IL-20, and IL-23 (31–35). TNF amplifies many of these responses, further activating DC populations in the skin and inducing cytokines, such as IL-1, IL-6, and IL-8, from keratinocytes and fibroblasts to promote continued Th17 differentiation and neutrophil recruitment. Neutrophils, in turn, can cause tissue damage via reactive oxygen species and proteases, which exposes self-Ags and generates endogenous TLR ligands. TLR stimulation of skin-resident DCs causes IL-12 synthesis, which can promote Th1 development, IFN-γ production, and generation of CXCR3 chemokines that recruit more T cells to the lesion (36). There may be a role for IFN-γ in maturation of DCs, particularly at plaque initiation (6). Known triggers for psoriasis include trauma and infection, resulting in generation of exogenous or endogenous TLR ligands and other pathogen-associated and damage-associated molecular patterns, which may provide entry points into the cycle of mutually reinforcing gene expression and cell-recruitment loops described above.

The IL-1 family contains 11 members (37, 38). IL-1α, IL-1β, IL-18 and IL-33 all have known roles as agonists affecting inflammation and/or adaptive immunity, whereas IL-1ra acts to inhibit...
IL-1α and IL-1β action. IL-1F6, -1F8, and -1F9 are agonists of the IL-1R family member IL-1RL2 (also known as IL-1Rrp2) (39, 40). IL-1F5 serves to antagonize these responses in a manner parallel to that used by IL-1ra for IL-1 responses (39) (J.E. Towne, B.R. Renshaw, J. Douangpanya, B.P. Lipsky, M. Shen, C.A. Gabel, J.E. Sims, unpublished data). Global-expression analysis of IL-1 family members demonstrated that IL-1F6, -1F8, and -1F9 were highly expressed in only a few tissues (39, 41–43) (data not shown). Among these, the most abundant expression was in skin. We surveyed a variety of human inflammatory skin conditions and found that IL-1F6, -1F8, and -1F9 were upregulated in psoriatic lesions (44) (H. Blumberg, H. Dinh, D.Shows, and J.E. Sims, unpublished data). Microarray studies of psoriatic skin also found upregulation of IL-1F9 (45).

We previously reported that transgenic overexpression of IL-1F6 under control of the keratin-14 promoter leads to an inflammatory skin phenotype at birth, which resolves after 2–3 wk of age. A number of inflammatory cytokines and chemokines are upregulated in the inflamed skin, and TNF inhibition leads to a decrease in epidermal thickness. In this article, we demonstrate that application of an irritant can rapidly induce psoriatic-like skin inflammation in phenotypically normal skin from 2–3-mo-old transgenic mice. We characterize the skin changes and cytokine involvement in much more detail than in our previous publication. We show that cytokines, such as IL-17, IL-22, and IL-23, known to be involved in human psoriasis, are overexpressed in this model and that they can induce IL-1F6, which, in turn, can induce IL-17, IL-22, and IL-23, thus establishing a self-amplifying gene-expression loop. We also provide a direct link to human psoriasis by demonstrating that agents approved for clinical treatment of psoriasis are beneficial in this model, as well as by showing that inhibition of the IL-1F6 receptor IL-1RL2 ameliorates the lesion phenotype in human psoriatic skin. The results presented in this article greatly strengthen the connection between IL-1RL2 ligands and human psoriasis.

Materials and Methods

Mice

Transgenic mice (44) were backcrossed at least eight times to C57BL/6 or FVB mice (both from Taconic Farms, Oxnard, CA). Male mice on the C57BL/6 background were further bred to female rag2\(^{-/-}\) mice (B6.129S6-Rag2\(^{tm1Fwa}\)N12; Taconic Farms), and backcrosses between those progeny were performed to create B6. \(\times\) rag2\(^{-/-}\) mice. Animal studies were conducted under protocols approved by the Institutional Animal Care and Use Committee at Amgen.

Protein reagents

Anti-mouse IL-1RL2 Ab (M616) is a rat IgG2a, and anti-human IL-1RL2 Ab (M146) is a mouse IgG1. Both were generated at Amgen. Soluble TNFRp75-Fc (etanercept) and a comparable protein made from murine IgG1 and murine TNFRp75 were from Amgen. Anti–IL-1β-23p19 mAb 16E5 (generated at Amgen) and the widely used anti–IL-12/23p40 mAb clone C17.8 were converted to a murine IgG1 isotype. 16E5 and C17.8 inhibit IL-23 induction of IL-17 in a murine splenocyte assay with comparable potency. 16E5 does not affect IL-12 induction of IFN-γ in mouse splenocytes, whereas the C17.8 Ab antagonizes IL-12 activity with comparable potency to its antagonism of IL-23. Murine IL-1F6 used for injection was an N-terminal truncation mutant antagonist of IL-12 activity with comparable potency to its antagonism of IL-1.

Skin transplantation and treatment protocol

Replicate 6-mm punch biopsies of full-thickness plaque skin were obtained from human skin donors with psoriasis. Sun-protected skin from nonpsoriatic donors was used as tissue recipients. One piece of tissue from each normal or psoriatic volunteer was transplanted onto the dorsal surface of a recipient mouse as follows. After the animal was anesthetized, the dorsal surface of the mouse was shaved. Mouse skin was surgically removed to size and replaced with the human tissue. This tissue was secured to the back of the mouse with absorbable sutures (4-0 Dexon “S”, Davis-Geck, Manati, Puerto Rico). The transplant was bandaged with Xeroform petrolatum dressing (Kendall, Mansfield, MA) for 5 d. The animals were maintained in a pathogen-free environment throughout the preparation and treatment phases. Treatment was initiated 1–2 wk posttransplantation, depending on the healing rate of the transplanted tissue. Animals with the human skin transplants were divided into treatment groups (isotype control Ab, anti–IL-1RL2 Ab, or etanercept as a positive control). Animals were treated with seven injections each of 150 \(\mu\)g anti-human IL-1RL2, isotype matched control Ab, or etanercept i.p. on alternate days. All procedures involving animals were approved by the University of Michigan Committee on Use and Care of Animals. At the end of the treatment phase, animals were photographed and then euthanized. The transplanted human tissue along with the surrounding mouse skin was surgically removed and fixed in 10% formalin. After embedding the tissue in paraffin, multiple 5-\(\mu\)m sections were cut from each tissue piece (~50 \(\mu\)m between sections), mounted onto microscope slides, and stained with H&E.

Immunohistochemistry

For cytoketeratin 6 and CD3 immunohistochemistry (IHC), skin sections were fixed in 10% neutral buffered formalin and embedded in paraffin. Deparaffinized tissue sections were subjected to Ag retrieval using Citra solution (no. HK086-9K; BioGenex, San Ramon, CA) in a Decloaking Chamber (Biocare, Concord, CA). Tissue sections were incubated with a 1:500 dilution of the cytoketeratin 6 Ab (no. PRB-169P; Covance, Berkeley, CA) or with a 1:200 dilution of the CD3 Ab (no CP215c; Biocare) at room temperature for

Downloaded from http://www.jimmunol.org/ by guest on August 19, 2021
60 min. Detection was performed with an anti-rabbit Mach 3 Rabbit AP Polymer Kit (nos. RP531L & RAP533L; Biocare), followed by Permanent Red chromogen solution (no. K0640; Dako North America, Carpinteria, CA). For the CD11c IHC, skin samples were frozen in OCT Compound (no. 4583; Sakura, Torrance, CA). Tissue sections were incubated with a 1:100 dilution of a biotinylated CD11c Ab (no. 553800; BD Biosciences, Pharmingen, San Diego, CA) at room temperature for 60 min. Detection was performed with streptavidin-alkaline phosphatase (no. NEL750; PerkinElmer, Waltham, MA) with tyramide signal amplification (no. SAT700B; PerkinElmer), followed by Permanent Red solution. For the CD31 IHC, skin samples were fixed in IHC zinc fixative (no. 550523; BD Biosciences, Pharmingen) and embedded in paraffin. Deparaffinized tissue sections were incubated with a 1:30 dilution of CD31 Ab (no. 533370; BD Biosciences Pharmingen) for 60 min at room temperature. Detection was performed with a Vectastain ABC-AP kit (no. AK-5000; Vector Laboratories, Burlingame, CA), followed by Permanent Red solution. Following the IHC-staining procedure, slides were counterstained with hematoxylin (no. S3309; Dako North America), dehydrated, cleared, and coverslipped. To quantify changes in the number of CD3+ and CD11c+ cells and the area of CD31+ vessels in response to TPA treatment, five representative digital images of each IHC assay from each individual animal were analyzed using MetaVue morphometry software (version 6.2r6; Universal Imaging, Downingtown, PA). The CD3 and CD11c IHC images were taken with an ×20 microscope objective (×200 magnification) and are expressed as the number of positive cells per mm². The CD31 images were taken with an ×40 microscope objective (×400 magnification) and are expressed as the CD31+ area in μm²/mm² of dermis evaluated.

Results

We previously reported that transgenic overexpression of IL-1F6 in mouse skin results in a hyperproliferative, inflammatory skin condition in newborn animals that resolves by 3 wk of age only to reappear at ~6 mo (44). Resolution could be prevented and the phenotype exacerbated by eliminating one copy of the IL-1F5 an-
agonist gene (44). We have now found that treatment of transgenic mouse skin with TPA at a time when it is phenotypically normal (∼2–3 mo of age) elicits skin inflammation to a much greater extent than TPA treatment of nontransgenic skin (Fig. 1A, 1B, Supplemental Fig. 1A). The morphology of TPA-treated transgenic skin is similar to lesional skin of human psoriasis. Macroscopically, the skin appears reddened, thickened, scaly, and crusty (Fig 1A). Histologically, there is epidermal hyperplasia (acanthosis); a thickened stratum corneum (hyperkeratosis) containing nucleated cells (parakeratosis); neutrophilic microabscesses in the stratum spinosum and the stratum corneum; a mixed dermal infiltrate containing macrophages/DCs, neutrophils, and lymphocytes; and an increase in and dilation of superficial dermal blood vessels (as evidenced by CD31 staining, which is increased 6-fold in TPA-treated transgenic mice compared with 1.8-fold in TPA-treated wild-type mice) (Fig. 1B, 1C, Supplemental Fig. 1A, 1B, 1D). A granular layer (normally difficult to see in mouse skin) becomes prominent in the hyperplastic skin of TPA-treated wild-type mice but is mostly lost in TPA-treated transgenic mice, particularly in areas of more severe lesion. Although the most obvious epidermal penetrations into the dermis are associated with hair follicles, there is more unevenness to the lower margin of the epidermis than is typical for mouse, suggestive of rete ridges found in psoriatic skin. Cytokeratin 6, indicative of proliferating keratinocytes, is expressed throughout the epidermis (Fig. 1D) in wild-type and transgenic mice in response to TPA. However, it is noteworthy that even in apparently normal untreated transgenic mice, cytokeratin 6 staining reveals focal patches of mildly proliferative skin (Fig. 1D, Supplemental Fig. 1C), suggesting that the transgenic skin is poised on the brink of abnormal proliferative changes. These focal proliferative patches are not seen in wild-type mice. CD3+ T cells are also increased in the epidermis after TPA treatment in transgenic and wild-type mice (2.3-fold and 1.8-fold, respectively) (Fig. 1E, Supplemental Fig. 1D). There is a significant increase in CD11c+ DCs, especially in the upper layers of the dermis (9.7-fold in transgenic mice, 2.7-fold in wild-type mice after TPA treatment) (Fig. 1F, Supplemental Fig. 1D). An excess of DCs over T cells, as seen in the transgenic mice after treatment with TPA, is typical of psoriasis (46). Although the general presentation is similar to that of human psoriatic skin, there are also points of difference. The most obvious of these is the role of T lymphocytes. Disease in transgenic mice occurs on a lymphocyte-deficient rag2−/− background (Fig. 1G), suggesting that T cells are not required, whereas T lymphocytes are believed to play a key role in human psoriasis. Other differences include the variable presence or loss of a granular-appearing layer beneath the stratum corneum, the relative paucity of lymphocytes in the infiltrate in the mouse disease, and the uncertain presence of rete ridges. Elicitation of skin hyperplasia and inflammation by TPA is dependent on the IL-1F6 transgene, because it can be prevented by injection of an anti–IL-1RL2 neutralizing mAb (Fig. 1H). These findings made us wonder whether IL-1F6, -1F8, and/or -1F9 might account for part of the epidermal contribution to human psoriasis.

Gene-expression patterns

To probe the similarity between human psoriasis and skin inflammation caused by overexpression of IL-1F6, we examined the expression of a number of genes relevant to skin or inflammation by quantitative PCR. Skin samples were taken from nontransgenic and K14/F6 transgenic mice, either untreated or treated with TPA. Genes substantially upregulated in transgenic animals treated with TPA treatment results in elevated expression of genes encoding cytokines and antimicrobial peptides in K14/F6 mouse skin treated with TPA.

FIGURE 2. TPA treatment results in elevated expression of genes encoding cytokines and antimicrobial peptides in K14/F6 mouse skin treated with TPA. Mice were treated with TPA or acetone vehicle on days 0 and 4 or were not treated (naive), and RNA was prepared from full-thickness skin sections harvested 4 or 48 h after the second TPA injection. TaqMan quantitative PCR was performed, and the cycle threshold (CT) value for each gene was normalized to that for HPRT. The normalized values for selected genes are shown. Each data point represents one mouse. The figure is representative of two experiments.
TPA, but not in other groups, included cytokines, chemokines, and antimicrobial peptides that are known to be upregulated in psoriasis (Fig. 2, Supplemental Table 1). These include IL-17A, IL-22, IL-23 (both p19 and p40 subunits), and the antimicrobial peptides S100A8, S100A9, and β-defensin 4 (the ortholog of human β-defensin 2). IL-1β, a regulator of Th17 differentiation, was also upregulated, as were IL-19, IL-20, and IL-24, cytokines related to IL-22 and possessing similar activities. TNF-α was upregulated by TPA in transgenic and nontransgenic mice.

**Mutual induction of inflammatory cytokines and IL-1 family proteins**

To establish directly whether IL-1F6 is capable of inducing genes implicated in psoriasis pathology, we injected IL-1F6 intradermally into wild-type mice and analyzed transcript levels in skin for selected genes (Fig. 3A, Supplemental Table 2). IL-1F6 led to substantial increases in IL-17A, IL-23, TNF, and IFN-γ mRNA. IL-1F6 also strongly induced itself, as well as other IL-1 family members, chemokines, growth factors, and antimicrobial proteins.

We next asked whether cytokines known to be elevated in psoriatic skin could induce IL-1F6 and other family members. TNF, IL-17A, IL-23, and IFN-γ were able to induce IL-1F6 mRNA following intradermal injection of cytokines into wild-type mice (Fig. 3B, Supplemental Table 3, data not shown). Induction was usually stronger when combinations of these cytokines, as well as IL-22, were used (Supplemental Table 3). Some of these cytokines also induced IL-1F8 and IL-1F9 (Supplemental Table 3). Thus, a positive feedback loop may exist in psoriatic skin, with cytokines, such as IL-17, IL-22, IL-23, and TNF, inducing IL-1F6 and IL-1F9, which, in turn, amplify expression of the previously implicated inflammatory cytokines as well as themselves.

**Response to therapeutics**

TNF inhibitors are effective in treating psoriasis (47) and are approved for this purpose in many countries. Abs that block the action of IL-12 and IL-23 have proven successful in clinical trials (48), and one is approved for treating psoriasis in the European Union, Canada, and the United States. Human-expression data and genetic-susceptibility studies suggest that Abs inhibiting the action of IL-23 alone would be effective as well (16, 49, 50). Because the morphologic appearance of and gene-expression patterns in the skin of K14/F6 transgenic mice treated with TPA were similar to those of human psoriatic skin, we asked whether agents known or suspected to be efficacious in treating psoriasis would have therapeutic effects in the mice. K14/F6 transgenic mice were treated with candidate agents on days −1 and 3 and with TPA at days 0 and 4. Mouse skin was evaluated macroscopically and histologically at day 6, and samples were taken for mRNA and protein analysis at 48 h after the second TPA application. Therapeutic agents examined were soluble TNFRp75-Fc, an anti–IL-12/23p40 Ab, and an anti–IL-23p19 Ab. Mice treated with any of these agents showed a markedly improved appearance of the skin (Fig. 4). The skin was also improved histologically, with reduced acanthosis, hyperkeratosis, parakeratosis, inflammatory cell infiltrate, dilation of dermal capillaries, and loss of granular layer compared with skin from mice treated with isotype-matched control Abs (Fig. 4). Moreover, gene-expression studies (Fig. 5, Supplemental Table 4) and protein analyses (Supplemental Table 5) showed reductions in mRNA for IL-17A and -17F, IL-22, IL-1β, chemokines, and antimicrobial peptides after treatment, as well as reductions in protein levels for most of these as well (IL-17A protein was below the level of detection for the assay used, and protein levels were not analyzed for antimicrobial proteins). For all of these features, inhibition of IL-12/23p40 or IL-23p19 was more effective than TNF inhibition, although the...
effectiveness of TNF inhibition varied from modest to excellent in different experiments. These results indicate that the psoriasis-like pathology in the skin of TPA-treated transgenic mice is responsive to the same therapeutic approaches used effectively for human psoriasis.

Mouse and human skin are quite different. Therefore, many investigators have studied psoriasis preclinically by transplanting skin from psoriasis patients onto immunodeficient mice (5, 6). Technical limitations, especially the small amount of tissue available, make this...
model unsuitable for biochemical studies, but it has been very informative when analysis is focused on efficacy as defined by histological parameters, such as epidermal thickness. Agents that are effective at treating psoriasis normalize the appearance of lesional human skin in these models (3, 4, 51, 52). We treated human psoriatic lesional skin transplanted onto SCID mice with an isotype control Ab or an Ab to the human IL-1RL2 receptor to block the action of IL-1F6, -1F8, and -1F9. As can be seen in Fig. 6, anti-IL-1RL2 substantially reduced the epidermal hyperplasia and other skin changes associated with psoriasis. In one experiment, we obtained enough tissue to also use etanercept as a positive control; the effects with etanercept were comparable to those seen with anti-IL-1RL2. The anti-human IL-1RL2 Ab does not cross-react with mouse IL-1RL2, and mouse IL-1F6 (or IL-1F8 or IL-1F9) does not signal through human IL-1RL2 (data not shown). Therefore, human IL-1F6, -1F8, and/or -1F9 are important for maintaining the psoriatic phenotype in the transplanted human skin in this model.

Discussion

Although psoriasis was originally thought to be a disease of the skin, much of the focus in the last decade has been on the role played by cells of the hematopoietic system, as well as their products, in driving the disease (1, 2). The contribution made by psoriatic skin to the disease has been less well explored. The evidence presented in this article suggests that IL-1F6, -1F8, or -1F9, acting through the IL-1RL2 receptor, may represent one important way in which keratinocytes contribute to psoriasis. We found that TLR3 ligands and inflammatory cytokines are able to induce IL-1 family members from human keratinocytes (H. Dinh, U. Martin, C.A. Gabel, J.E. Sims, unpublished data). Polymorphisms in the epidermal-differentiation complex on human chromosome 1 are associated with susceptibility to psoriasis (8, 12, 13), and it is possible that altered barrier function in skin allows microbial, osmotic, or other stimulators of keratinocytes to lead to production of IL-1F6, -1F8, and/or -1F9. The IL-1 family members could then induce expression of many genes capable of driving the phenotypic characteristics of psoriatic skin (IL-17, IL-22, IL-23, TNF-α) or of recruiting and maintaining the required cell populations (chemokines and antimicrobial proteins). The induced inflammatory cytokines are also capable of inducing more IL-1F protein, thus perpetuating the cycle.

The biggest difference between the skin inflammation in this mouse model and that in human psoriasis is the lack of dependence of the mouse disease on T cells. T cells are thought to be central to psoriasis pathogenesis, and cytokines that act on (IL-23, IL-12, and TNF) or are made by (IL-17, IL-22, TNF, and IFN-γ) T cells play important roles in disease. Although T cells are not required for the mouse skin inflammation, we have not investigated whether the disease mechanisms and gene-expression profiles are the same in wild-type versus rag2 knockout animals. In the wild-type background, at least, the IL-1F6 transgenically expressed in keratinocytes likely acts in an autocrine fashion on keratinocytes themselves as well as on skin DCs and macrophages; all of these cell types express the receptor protein IL-1RL2 (J.E. Sims et al., unpublished data). IL-1F6 may enhance synthesis of cytokines, chemokines, and antimicrobial proteins from keratinocytes and TNF, IL-20, and IL-23 from TIP-DCs (Fig. 3A, Supplemental Table 2). IL-1F6 can also assist in Th17 differentiation in vitro, substituting for IL-1 (albeit less potently) (D. Swart and J. Tocker, personal communication), leading to increased IL-17 production (Fig. 3A, Supplemental Table 2). Thus, forced IL-1F6 expression would simply be a different entry point to that provided by activated T cells for the upregulation of a cytokine network similar to that found in psoriasis. In the absence of T cells, it is not known whether other cytokines, such as TNF, IL-20, and IL-24, and IL-1F proteins can substitute for the key functions of IL-17 and IL-22 or whether IL-17 and IL-22 might still be present but made by other, non-rag2-dependent cell types. It is notable that mast cells can make IL-17 (53), that neutrophils contribute IL-17 during Leishmania infection (54), and that NK cells and DCs can express IL-22 (55, 56). In addition, IL-17 is induced, and IL-23 is critical for disease, in two T cell-independent colitis models (57, 58).

The mechanism by which TPA induces disease in K14/F6 mice is not understood. Although IL-1F6 mRNA expression is considerably higher in TPA-treated transgenic skin than it is in TPA-treated wild-type skin (Supplemental Tables 1, 6), it is induced only 3.5-fold by TPA in the transgenic skin, and the F5:F6 antagonist:agonist mRNA ratio is reduced by only 2-fold after TPA induction in the transgenic mouse (Supplemental Table 6). Changes in expression of other IL-1 family genes after TPA treatment are comparably modest. These small changes seem unlikely to account for the marked change in skin phenotype that follows TPA treatment of transgenic mouse skin. We recently found that the sp. act. of IL-1F6 is increased ~10,000-fold by proteolytic processing near the N terminus (J.E. Towne, B.R. Renshaw, J. Douangpanya, B.P. Lipsky, M. Shen, C.A. Gabel, J.E. Sims, unpublished data). TPA is known to induce expression of a large number of genes in mouse skin (Supplemental Table 1) (1), including proteases. It is possible that among these are genes required for activation and secretion of IL-1F6. Alternatively, TPA leads to an influx of macrophages, neutrophils, and T cells into skin (59). One or more of these cell types or their products might be important to complement the action of IL-1F6 in setting up the self-amplifying gene-expression loop. In this scenario, the preconditioning of the K14F6 transgenic mouse skin to perturbation by TPA would be regarded as analogous to the preconditioning of nonlesional psoriatic skin to respond to trauma in the well-known Koebner reaction.

Transgenic overexpression or knockout of other IL-1 family members in skin has led to various inflammatory conditions (60–62), but none show the many points of similarity to human psoriasis that are seen with overexpression of IL-1F6. Skin conditions resembling psoriasis to varying extents have also been created by manipulation of a number of other genes in mice (3, 4, 63). However, mouse models are perhaps less successful at reconstructing human disease than they are at allowing exploration of genes and pathways plausibly relevant to human conditions. TPA-treated K14/F6 transgenic mice develop a skin inflammation characterized by increased expression of many genes characteristic of human psoriasis, which is ameliorated by agents known to work clinically in psoriasis patients (TNF and IL-12/23p40 inhibitors). Amelioration by an anti–IL-23p19 Ab suggests that IL-23, rather than IL-12, is the dominant p40-containing cytokine involved in the mouse disease. Ligands for IL-1RL2 are also critical for maintaining the psoriatic characteristics of human lesional skin, at least when transplanted onto...
immunodeficient mice. The data presented in this article suggest that agents that block signaling through IL-1R2 could be useful therapeutically in psoriasis.

Acknowledgments

We thank Blair Renshaw for providing IL-1F cytokines, Hiko Kohno for murine TNFRFp75-Fc protein, Brian Lipsky for help with TDLA gene profiling, Kathy Rohrbach for help with IHC, Jacques Peschon for useful discussions, Eva Gonzalez-Suarez for advice on TPA treatment of mouse skin, and Guang Chen for help with analysis of gene-expression data, and Dirk Smith for comments on the manuscript.

Disclosures

H.D., C.D., E.S.T., K.B., D.S., J.E.T., and J.E.S. are employees of Amgen and own Amgen stock. N.B., N.D.A., and J.V. have no financial conflicts of interest.


