Cytokines as potential therapeutic targets for inflammatory skin diseases

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ABSTRACT. A network of pro-inflammatory cytokines is a central feature in the pathophysiology of cutaneous inflammatory diseases. Thus, the delineation of precise roles for particular cytokines and the development of cytokine-directed therapeutics have become areas of intense investigation. While anti-TNF therapeutics have proven to be effective for the treatment of psoriasis, clinical investigations have now begun with other cytokine-directed therapies, such as those targeting IFN-g, IL-12p40, and IL-18. In addition to therapeutics that target cytokines directly, strategies that target cytokine signaling pathways are in development too. In this short review, we summarize key findings from a recent workshop on cytokines as potential therapeutic targets for inflammatory skin diseases.

Keywords: cytokines, inflammation, psoriasis

A unifying model for the pathophysiology of psoriasis, with cytokines as the central mediators, was described by Nickoloff in 1991 [1]. While this cytokine network theory of psoriasis proposed the pathological involvement of multiple cytokines, including TNF-α, the relative importance of TNF-α only became apparent with subsequent experimental investigations [2]. The hypothesized key role for TNF-α in psoriasis and other inflammatory diseases led to the development of therapeutic strategies targeting TNF-α specifically. A number of soluble TNF receptors and anti-TNF antibodies have now been either approved by the FDA for the treatment of psoriasis or are in the late stages of development.

The pendulum though, has now swung back towards a greater interest, not only in TNF-α, but also the other cytokines responsible for the initiation, maintenance, or resolution of psoriatic skin inflammation *(table 1)* and other inflammatory dermatoses that may be considered as "visible immunological diseases". A workshop sponsored by Berlex Biosciences held in the Napa Valley, California, November 17-19, 2004 focused on cytokines (including, but not limited to, TNF-α) as potential therapeutic targets for inflammatory skin diseases. The current report summarizes key findings presented at the workshop. These proceedings will be published by Springer-Verlag (Berlin/Heidelberg, Germany).

Table 1
Cytokines in psoriasis

Cytokines	Function	Presence in psoriatic plaques[a]
TNF-α, IFN-γ, IL-2, IL-6, IL-8, IL-12p40, IL-15, IL-17, IL-18, IL-1F6, IL-1F8	Pro-inflammatory	Elevated
IL-4, IL-10, IL-1RA	Anti-inflammatory	Reduced

[a] mRNA or protein.

PRO-INFLAMMATORY CYTOKINES APART FROM TNF-α, AS NOVEL TARGETS – LESSONS FROM CLINICAL TRIALS

Gerald Krueger (University of Utah, Salt Lake City, UT, USA) set the stage for the workshop by summarizing the current state of clinical investigations involving anti-cytokine strategies. The soluble TNF receptor etanercept, the first anti-TNF therapeutic approved by the FDA for psoriasis, represents a major advancement, and appears to be safe and effective for many patients. The antibodies directed against TNF-α, infliximab and adalimumab, are likely to be approved by the FDA for psoriasis in the near future. Biologics in development, targeting other cytokines, include IL-18-binding protein, and anti-IL-12, anti-IL-18 receptor, and anti-IFN-γ monoclonal antibodies *(table 2)*. Gottfried Alber (University of Leipzig, Germany) discussed the IL-12 family including IL-12 and IL-23, which share a p40 subunit, and IL-27. He reminded the audience that current clinical studies targeting the p40 subunit, commonly referred to as "anti-IL-12 therapy", will not be able to differentiate between pathogenic effects derived from IL-12 (p35/p40) or IL-23 (p19/p40), and

Table 2
Anti-cytokine therapies in clinical development for psoriasis

Therapeutic	Mechanism	Stage of development
Etanercept	Soluble TNF receptor	Approved in US 04/04
Infliximab	Anti-TNF monoclonal ab	Phase III
Adalimumab	Anti-TNF monoclonal ab	Phase II completed
Onercept	Soluble TNF binding protein	Phase III
CNTO-1275	Anti-IL-12p40 monoclonal ab	Phase I/II
IL-18 bp	IL-18 binding protein	Phase II
Fontolizumab	Anti-IFN-γ monoclonal ab	Phase I completed

suggested that p19- and p35-knockout mice may be more useful for this purpose.
Boris Skurkovich (Brown University, Providence, RI, USA) presented clinical results using polyclonal anti-IFN-γ therapy in a variety of TH1-mediated skin diseases including psoriasis, alopecia areata, and vitiligo. Studies with a humanized monoclonal antibody directed against IFN-γ are also being pursued in clinical trials, but no data are presently available.
A rationale for anti-IL-15 clinical trials in psoriasis was presented by Ian McInnes (University of Glasgow, Scotland). IL-15 is elevated in psoriatic skin and a humanized anti-IL-15 antibody, AMG714, reduced inflammation and typical psoriatic skin features in human psoriatic skin grafted onto SCID mice. Anti-IL-15 antibodies are currently in Phase II trials for rheumatoid arthritis. However, all of these anti-cytokine strategies for treating psoriasis must also be compared to the new biologics targeting T cells directly, such as alefacept and efalizumab, which block T cell/antigen presenting cell interactions, as well as the non-biologics still in use [3].
A major impact of cytokines has also been demonstrated in several other inflammatory skin diseases such as allergic and atopic dermatitis. However, clinical intervention involving the cutaneous cytokine network has so far been focused mainly on psoriasis.

WHICH CYTOKINES ARE ON THE HORIZON AS POTENTIAL TARGETS?

A number of presenters reviewed the use of transgenic and knockout mice for identifying pro-inflammatory or anti-inflammatory activities of particular cytokines. Yochiro Iwakura (University of Tokyo, Japan) described the inflammatory disease-like conditions that develop spontaneously in mice deficient in IL-1-receptor antagonist. The development of a destructive, rheumatoid arthritis-like disease and aortitis was significantly suppressed by the additional deficiency of TNF-α or IL-17, whereas the development of dermatitis was inhibited by the simultaneous deficiency of TNF-α. These studies suggest a role for IL-1-induced TNF +/- IL-17 at particular sites of inflammation. John Sims (Amgen and University of Washington, Seattle, WA, USA) summarized findings using overexpression of IL-1 and IL-1R family members specifically in the skin. Skin-specific expression of IL-1α or IL-18, under the control of the keratin 14 promoter, leads to an inflammatory condition, and in the case of IL-18 it exacerbates the dermatitis elicited by contact hypersensitivity [4]. Transgenic skin-specific expression of two newer members of the IL-1 family, IL-1F6 or IL-1F8, or their putative common receptor IL-1R rp2 induces an inflammatory skin condition, which histologically bears some degree of resemblance to human psoriasis. Consistent with these findings, elevated levels of both IL-1F6 and IL-1F8 are found in psoriatic skin.

DENDRITIC CELLS, REGULATORY T CELLS, AND ROLES FOR ANTI-INFLAMMATORY CYTOKINES

Upon activation by microbes or cytokines, immature dendritic cells (DC) acquire the ability to present processed antigen and secrete cytokines, thus influencing the immune response and, *via* cytokines such as IL-12, the critical Th1/Th2 balance.
Alexander Rudensky (University of Washington, Seattle, WA, USA) discussed the regulation of antigen presentation by lysosomal cysteine proteases and pointed out the preferential use of cathepsin S by dendritic cells. While numerous cytokines are produced by activated DC, Paola Ricciardi-Castagnoli (University of Milano-Bicocca, Milan, Italy) identified IL-2 as an unexpected dendritic cell-derived cytokine that acts upon NK cells, thus linking DC to the innate immune response as well. Grant Gallagher (University of Medicine and Dentistry of New Jersey, Newark, NJ, USA) discussed a role for IL-19, a member of the IL-10 family, in inducing increased IL-10 in DC and shifting the Th1/Th2 balance towards the Th2 phenotype. As recently reported [5], elevated levels of IL-10 in a subset of DC may be important for the induction of type 1 regulatory T (Tr1) cells, a regulatory T cell subset characterized by the ability to secrete IL-10 and TGF-β, cytokines with well documented anti-inflammatory activities.
Unlike Tr1 cells, conventional $CD4^+CD25^+$ regulatory T cells do not secrete TGF-β, but exert their immunosuppressive function in a cell contact-dependent manner. Alexander Enk (University of Heidelberg, Heidelberg, Germany) demonstrated the generation of antigen-specific $CD4^+CD25^+$ regulatory T cells by targeting $CD11c^+$ DC with antigen, and Kevin Cooper (Case Western Reserve University, Cleveland, OH, USA) described a functional defect in $CD4^+CD25^+$ regulatory T cells in psoriatic patients. Richard Flavell (Yale University, New Haven, CT, USA) presented evidence that overexpression of TGF-β in T-cells expands the pool of $CD4^+CD25^+$ regulatory T cells, thus providing an additional mechanism for the immunosuppressive function of this cytokine.
As an alternative to the antibodies or soluble receptors directed against pro-inflammatory cytokines discussed above, the use of anti-inflammatory cytokines as therapeutics for inflammatory skin diseases remains a promising approach. In this regard, significant effects of IL-4 therapy in psoriasis have been reported [6]. More information, however, has been generated from clinical studies with recombinant human IL-10 [7]. After successful early trials, a larger study involving 28 patients with moderate-to-severe psoriasis showed that treatment with IL-10 resulted in only temporary clinical improvement despite sustained systemic decreases in pro-inflammatory cytokines [8]. Remarkably, IL-10 clearly failed in atopic dermatitis (Christian Reich, Goettingen, personal communication).

INTRACELLULAR SIGNALING PATHWAYS – AN ALTERNATIVE MEANS OF TARGETING PARTICULAR CYTOKINES

Antibodies directed against cytokines and recombinant cytokines have been extremely effective in defining roles, or the lack thereof, for specific cytokines in inflammation. The use of these reagents preclinically, also allows for easy transition into the clinic if warranted. However, protein-based therapeutics, particularly antibodies and soluble receptors, are expensive to produce, require sustained elevated blood levels in order to develop significant tissue (skin) levels, and must be administered by injection. Small-molecule, orally active therapies would be welcomed. Thus, once a cytokine has been validated as an important target, alternative means of suppressing (or inducing) that cytokine by manipulation of relevant signaling pathways have been sought. Charles Dinarello (University of Colorado, Denver, CO, USA) discussed the ability of histone deacetylase (HDAC) inhibitors, molecules initially identified as anti-cancer therapeutics, to reduce gene expression of a number of cytokines involved in inflammation. He also presented a number of animal models of inflammation in which the administration of HDAC inhibitors ameliorated disease. Bharat Aggarwal (University of Texas, Houston, TX, USA) summarized the current understanding of TNF-α signaling, and highlighted NFκB inhibitors as promising alternatives for TNF blockers.

CONCLUSIONS AND OUTLOOK

The rationale for targeting TNF-α in inflammatory skin diseases was based on a firm understanding of the biology of TNF-α derived from a great deal of experimentation. However, a central role for TNF-α in psoriatic disease was only truly defined after the completion of human clinical trials with specific neutralizing therapies. Clinical investigation has now begun for other, cytokine-directed therapies, such as those targeting IFN-γ, IL-12p40, and IL-18. IFN-γ, as a T cell-derived cytokine that activates macrophages and induces TNF-α and chemokine secretion, may be particularly well-suited for targeting. In addition to regulating the immune response, IFN-γ acts as a trigger for epidermal hyperplasia when injected into the skin, and may directly affect keratinocytes by increasing levels of Bcl-x and inhibiting apoptosis [9]. An additional T cell-derived cytokine that appears to be a promising target for inflammatory skin disease is IL-17, a mediator induced by IL-15 and IL-23, two other cytokine targets of interest. However, caution is needed since research once supported IL-8 as a central player in the pathogenesis of psoriasis prior to unremarkable clinical results with anti-IL-8 therapy [10]. Clinical studies still remain the key arbiter in determining the pathogenic relevance of a particular cytokine.

Although psoriasis is currently the primary dermatological disease indication for these new therapeutic approaches in clinical development, it is likely that some of them may be of value for other cutaneous and non-cutaneous inflammatory disorders. In addition, basic research into the molecular mechanisms of cytokine action will undoubtedly lead to the identification of novel targets, some of which may be more amenable for the development of orally active, small molecules that would be more suitable for the treatment of larger populations and more diverse types of cutaneous disease.

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