Therapeutic antibodies that target inflammatory cytokines in autoimmune diseases

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Received 29 September 2015, accepted 18 October 2015

Abstract

Inflammatory cytokines are key regulators of immune responses. Persistent and excessive production of inflammatory cytokines underscores the development of autoimmune diseases. Therefore, neutralizing inflammatory cytokines or antagonizing their receptor function is considered as a useful therapeutic strategy to treat autoimmune diseases. To achieve the success of such a strategy, understanding of the complex actions of these cytokines and cytokine networks is required. In this review we focus on four inflammatory cytokines—tumor necrosis factor α (TNFα), interleukin-6 (IL-6), IL-23 and IL-17—and dissect how the dysregulation of these cytokines regulates autoimmune diseases. On the basis of pre-clinical and clinical data, we specifically discuss the therapeutic rationale for targeting these cytokines and describe the potential adverse effects.

Keywords: autoimmune diseases, IL-6, IL-17, IL-23, T₁₇, TNFα

Introduction

Since interferon (IFN) was discovered in 1957 (1, 2), more than 90 inflammatory cytokines and their corresponding receptors have been identified (3, 4). These cytokines are produced by various cell types, and regulate immune responses, wound healing, angiogenesis, hematopoiesis and tissue remodeling. An appropriate inflammatory response is vital to host defense, whereas excessive or persistent production of inflammatory cytokines results in immunopathology such as autoimmune diseases.

Although inflammatory cytokines exert variable effects at different stages of different autoimmune diseases, not all of them are effective or promising targets for the treatment of these diseases. Here we outline some of the most effective treatments for autoimmunity including those targeting tumor necrosis factor α (TNFα), interleukin-6 (IL-6), IL-23 and IL-17, and discuss the underlying biological mechanisms. We also discuss the future challenges in the development of cytokine-targeted drugs on the basis of pre-clinical and clinical data.

Cytokines in autoimmune diseases

Upon infection or injury, keratinocytes, macrophages, dendritic cells (DCs) and other cells are activated to produce inflammatory cytokines (5–9). These inflammatory cytokines in turn act back on macrophages and DCs to induce more inflammatory cytokines, chemokines and other antimicrobial mediators (10–12). Subsequently, chemokines recruit myeloid DCs, neutrophils and T cells to the site of infection or injury to further amplify inflammatory responses to protect hosts from microbial infections or to repair tissue damage (13, 14). However, under certain circumstances, possibly due to the presence of autoreactive T cells in the case of autoimmunity, there is uncontrollable production of inflammatory cytokines. Work for the past 10 years has revealed a vital role of T helper 17 (T₁₇) cells in autoimmune diseases.

For example, in psoriasis, IL-6, together with TGFβ, induces naive CD4⁺ T cells to differentiate into IL-17-producing T cells (15–17), and the persistent production of TNFα and other inflammatory cytokines activates macrophages and keratinocytes to constantly produce chemokines such as CCL20 and CCL27 to recruit immune cells including myeloid DCs, neutrophils and T₁₇ cells to lesional skin of patients with psoriasis (18–22). Among these immune cells, DCs are further activated by TNFα to produce IL-23 (23, 24). IL-23, on one hand, directly activates a subset of IL-23-receptor-expressing macrophages and DCs, resulting in the production of inflammatory cytokines such as TNFα and IL-1 (25–27). On the other hand, IL-23 not only promotes T₁₇ cells to become highly pathogenic but also activates γδ T cells; both cell types produce IL-17A, IL-17F, IL-6 and TNFα (26, 28–30). IL-17 activates epithelial cells (e.g. keratinocytes), endothelial cells and fibroblasts to produce a variety of inflammatory cytokines (31–33), chemokines (34) and antimicrobial peptides/proteins (AMPs) (35–37).
In addition, IL-17 synergizes with other inflammatory cytokines including TNFα and IL-1β to further induce the expression of inflammatory cytokines and chemokines (38). The inflammatory cytokines induced by IL-17 promote the expansion of IL-17-producing γδ T or T17 cells whereas the chemokines recruit more neutrophils or IL-17-producing T cells to sites of inflammation in the skin (30, 39, 40); these processes result in a feed-forward mechanism to further amplify local inflammatory responses to incite cytokine storms, thus leading to the inflammatory manifestation of psoriatic skin (Fig. 1).

Taken together, the inflammatory circuit of TNFα, IL-6, IL-23 and IL-17 plays a critical role in host defense and tissue repair, whereas the dysregulation of this inflammatory circuit leads to the development of autoimmune diseases such as psoriasis.

Targeting inflammatory cytokines in autoimmune diseases

Given the importance of TNFα, IL-6, IL-23 and IL-17 in the development and pathogenesis of inflammatory and/or autoimmune diseases, lots of efforts have been spent on targeting these cytokines to treat these diseases. Accumulating pre-clinical and clinical studies show that blocking TNFα, IL-6, IL-23, IL-17 or their corresponding receptors by use of neutralizing antibodies is highly effective in the treatment of multiple autoimmune diseases such as psoriasis, rheumatoid arthritis (RA) and inflammatory bowel disease (IBD; Table 1). In particular, targeting the IL-23–IL-17 axis has been the most successful strategy for the treatment of psoriasis in the past decade.

Tumor necrosis factor α

TNFα is one of most avidly studied and clinically targeted cytokines in the treatment of autoimmune diseases. To date, TNFα blockers are still the best-selling cytokine-targeting drug type on the market. TNFα is expressed by several cell types including keratinocytes, macrophages, monocytes, neutrophils, and T lymphocytes while its receptors, TNF receptor 1 (TNFR1; also known as p60) and TNFR2 (also known as p75), are ubiquitously expressed. Deregulated production of TNFα and its TNFRs is detrimental and has been associated with sepsis and several other inflammatory and autoimmune diseases including RA, psoriasis and colitis.

In the early 1970s, TNFα was discovered as a potent muscle-wasting (or cachexic) factor with potent anticancer activity (41, 42). This discovery stimulated great enthusiasm in the treatment of cancer. Unexpectedly, the administration of TNFα to patients caused hypotension and hepatic damage (43). The failure of TNFα in cancer treatment made scientists shift their attention to the field of sepsis as macrophage-derived TNFα was found to be one of two primary inflammatory cytokines in the pathogenesis of septic or endotoxin shock (44). However, the inhibition of TNFα with neutralizing antibodies or soluble TNFα receptor in patients with sepsis was either ineffective or made survival outcomes worse (45, 46).

Although anti-TNFα therapies failed in the treatment of cancer and sepsis, the first clinical trial of a TNFα inhibitor succeeded in the treatment of RA in 1992 (47). This success subsequently led to five approved TNFα-blocking biologicals (Table 1)—infliximab, etanercept, adalimumab, certolizumab and golimumab (48). Infliximab is an IgG1 mouse–human chimeric antibody and was the first TNFα-targeted drug to be approved, in 1998 (49). This was followed by the approval of the TNFR2–Fc (crystallizable fragment of immunoglobulins) IgG1 chimera protein etanercept (50). Adalimumab was the first fully human antibody targeting TNFα and was approved in 2002 (51). Certolizumab pegol is a new, polyethylene glycol-conjugated, humanized Fc (antigen-binding fragment of immunoglobulins) of a TNFα-specific monoclonal antibody.

**Fig. 1.** The inflammatory circuit of TNFα, IL-6, IL-23 and IL-17 in the pathogenesis of psoriasis. The persistent production of TNFα and other inflammatory cytokines activates DCs to produce IL-23, and IL-6, together with TGFβ, induces naive CD4+ T cells to differentiate into IL-17-producing T cells. IL-23 directly activates macrophages (Mφ) to produce inflammatory cytokines such as TNFα and IL-1; IL-23 also promotes T17 cell differentiation into highly pathogenic T17 cells and activates γδ T cells, both cell types constantly produce IL-17A, IL-17F, IL-6 and TNFα. IL-17 activates epithelial cells (e.g. keratinocytes) and endothelial cells to produce a variety of inflammatory cytokines, chemokines and AMPs. The inflammatory cytokines induced by IL-17 promote the expansion of IL-17-producing γδ T or T17 cells whereas the chemokines recruit more neutrophils or IL-17-producing T cells to sites of inflammation in the skin, which produces a positive feed-forward mechanism and further amplifies local inflammatory responses to incite cytokine storms in psoriasis.
<table>
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<th>Biologic</th>
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<td>Infliximab (Remicade®)</td>
<td>Janssen Biologics (Philadelphia, PA, USA)</td>
<td>Chimeric TNFα-specific antibody</td>
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<td>Etanercept (Enbrel®)</td>
<td>AMGen (Thousand Oaks, CA, USA) / Pfizer (New York, NY, USA)</td>
<td>Human TNFR2-Fc fusion protein</td>
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<td>Adalimumab (Humira®)</td>
<td>Abbott Laboratories (Chicago, IL, USA)</td>
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<td>Certolizumab pegol (Cimzia®)</td>
<td>UCB (Brussels, Belgium)</td>
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<td>Human IL-12/IL-23-specific antibody targeting the p40 subunit</td>
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IL-6R form generated by proteolytic cleavage of membrane-bound IL-6R. Therefore, the antibodies directed against IL-6R α will target both membrane-bound and soluble forms of the receptor, which may be outweighed in some cases by adverse effects. In such cases, targeting signaling molecules such as JAKs downstream of IL-6R α might be more effective. Thus, tofacitinib, a small-molecule therapeutic targeting JAK1 and JAK3, has proved to be significantly effective (70). Moreover, IL-6 plays a critical role in host defense against microbial infections (71, 72) and plays a protective role during neural and liver injury (73, 74). Long-term IL-6 neutralization may increase susceptibility to bacterial and viral infections or may cause mortality in patients with ischemic neural damage or alcoholic cirrhosis.

The IL-23–IL-17 axis

The discovery of the IL-23–IL-17 immune axis has led to defining a new lineage of T helper cells—T17 cells. This new T-cell subset plays a key role in the pathogenesis of multiple autoimmune diseases as T17 cells are one of major producers of IL-17 (75).

Interleukin-23

IL-23 is a heterodimeric cytokine composed of the p19 and the p40 subunits, and shares the p40 subunit with IL-12, which contains another subunit—p35. Given its key role in driving pathogenic T17 and γδ T cells to produce high levels of IL-17 (Fig. 1), neutralizing antibodies have been developed that target IL-23. The anti-p40 subunit therapy targeting IL-12 was first evaluated in patients with Crohn’s disease (CD) in 2000 and provided benefit in this disease (76). Subsequent studies demonstrated that, in patients with psoriasis, levels of mRNA for the p40 of IL-12 or IL-23 and for the p19 of IL-23 were higher in lesions of psoriasis patients than in non-lesional and normal skin, whereas mRNA for p35 of IL-12 was present but decreased in lesional skin (77). Moreover, pre-clinical models of psoriasis illustrated that IL-23 exposure in murine skin drove the excessive growth and abnormal differentiation of keratinocytes, whereas IL-12 did not promote the same pathology (77, 78).

The evidence suggests that targeting the p40 of IL-12 indeed inhibits IL-23 and that inhibiting p40 might be effective to treat psoriasis, too. Therefore, two therapeutic mAbs targeting the p40 subunit, Ustekinumab and briakinumab, were used in treatment of psoriasis (Table 1). Both Ustekinumab and briakinumab were effective in Phase III clinical trials for the treatment of psoriasis and psoriatic arthritis (4). Ustekinumab also showed benefits in the treatment of moderate to severe CD when it was administered as maintenance therapy (79), but failed to prevent inflammation in patients with MS (80).

To date, Ustekinumab has been approved to treat psoriasis and psoriatic arthritis (81). However, briakinumab was withdrawn pending further analysis as major adverse cardiac events occurred in a higher number of patients receiving briakinumab compared with those receiving placebo in some Phase III trials (81, 82). Besides Ustekinumab and briakinumab, several IL-23-specific antibodies antagonizing the p19 subunit including tidikizumab, guselkumab,
LY2525623, AMG139, BI-655066 and LY3074828 (Table 1) have been developed (81). Tildrakizumab and guselkumab have completed Phase II trials for psoriasis. LY2525623 was terminated in Phase II for complexities in development but not safety concerns. AMG139, BI-655066 and LY3074828 are in early stages of development (81).

Although IL-23 is redundant in host defense to many pathogens, it is important for host responses against Mycobacterium and Candida infections as IL-23-dependent IL-17A and IL-17F play key roles in protecting hosts from these infections (83, 84). Therefore, treatment of autoimmune diseases with IL-23 antagonists carries the risk of impaired host defense responses to pathogens. Moreover, IL-12 can act on both innate and adaptive lymphoid cells such as natural killer cells and CD8+ cytotoxic T lymphocytes, and these cells then produce IFNγ to prevent tumor initiation, growth and metastasis (85, 86). In contrast, IL-23 increases tumor cell proliferation, survival and invasion by activating signal transducer and activator of transcription 3 (STAT3) and the overexpression of IL-23 in mice is sufficient to induce rapid (3–4 weeks) de novo development of intestinal adenomas (85, 87, 88). Therefore, the risk of breakdown in tumor surveillance by antagonizing the IL-12/IL-23 p40 subunit is of particular concern. To achieve both efficacy and safety in the treatment of autoimmune diseases by targeting IL-23, the intricate cellular and molecular mechanisms of those diseases warrant further investigation. Meanwhile, clinical testing is required to determine whether a specific disease mechanism also operates in humans.

**Interleukin-17.** As a key effector cytokine produced by pathogenic T17 and γδ T cells, IL-17 plays a crucial role in the pathogenesis of multiple autoimmune diseases such as psoriasis, RA, MS, IBD and myocarditis (89–91) and has been thought as one of the best targets in the treatment of autoimmune diseases (92). Therefore, several monoclonal antibodies targeting IL-17A and IL-17RA have been developed (Table 1). The Phase II proof-of-concept studies for secukinumab (a fully human IL-17A-specific monoclonal antibody) (93), ixekizumab (a humanized IL-17A-specific antibody) (94) and brodalumab (a fully human IL-17RA-specific monoclonal antibody) (95) showed that each drug was highly effective and helped around 80% of treated patients to achieve a 75% reduction in the Psoriasis Area and Severity Index (PASI) (96).

To date, secukinumab has been approved for the treatment of plaque psoriasis, whereas ixekizumab and brodalumab are in Phase III trials (92). Beyond psoriasis, secukinumab, ixekizumab and brodalumab are currently in pipeline, with trials in psoriatic arthritis and RA still ongoing. Other drug candidates targeting IL-17A include CNT06785, SCH-900117 and bimekizumab. All these candidates are in either Phase II or Phase I clinical trials (97).

Although both IL-17A and IL-17RA are good targets in the treatment of autoimmune diseases, a key question remains: targeting which one—the cytokine or the receptor—is more effective? From a pharmacokinetic point of view it may be easier to block the cytokine than the receptor. IL-17 is readily available in the circulation, whereas the receptor resides in the membrane of many cell types and the corresponding neutralizing antibodies need to gain access to the relevant tissue before the receptor can be neutralized (92). Therefore, it is reasonable to speculate that targeting the ligand would be more straightforward than the receptor. However, IL-17RA is the most common signaling subunit in the IL-17 pathway. It can form heterodimeric receptor complex with most other IL-17 receptors. Therefore, targeting IL-17RA might be an effective way to disrupt the pathway if multiple IL-17 cytokines are contributing to a disease rather than a particular cytokine. Recent positive results for brodalumab in Phase III trials in psoriasis have confirmed this hypothesis (97).

Similar to targeting IL-23, the rates of adverse events including infections need to be considered due to a crucial role of IL-17A in the control of extracellular but not intracellular bacterial infections and fungi (98). To avoid the adverse effects, blocking T17 cell responses but not universal IL-17 cytokine signaling might be safer. Therefore, targeting RORγt, a key transcription factor that determines the lineage commitment of T17 cells, might be an alternative option. Several small molecules including digoxin and ursolic acid have been developed to antagonize RORγt activity (99, 100). However, off-target effects of these small molecules must be under key consideration.

**Conclusions**

Over the past two decades, targeting inflammatory cytokines and their receptors has produced multiple highly successful drugs in the treatment of autoimmune diseases such as RA, psoriasis and colitis. Although the potential of inflammatory cytokines and their receptors as drug targets is clearly established, how to select the key players in specific autoimmune diseases or pivotal regulators of cytokine circuits is still under debate. Therefore understanding the intrinsic cellular and molecular mechanisms of each cytokine in different disease settings is required to select a specific inflammatory target with better efficacy and safety profiles in the treatment of each autoimmune disease. Additionally, although the general principles in defining good anti-inflammatory targets are well established (3), the redundancy in the actions of many inflammatory cytokines that will limit the efficacy of single neutralizing agents requires us to pay careful attention.

We should point out that not all agents work as expected; some work better than others in certain diseases. Thus, the heterogeneity and complexity of human autoimmune diseases needs to be considered. Since mouse models cannot exactly resemble all types of human diseases, innovative translational studies on human patients are urgently required.

In addition, we need to keep in mind that inflammatory cytokines are also critical to maintain effective host defense or prevent tumorogenesis. Therefore, blocking cytokines to prevent autoimmune diseases might make patients more susceptible to infection or risk causing decreased tumor surveillance.

Clearly, there is much to be done to dissect the complex and highly interactive effects of inflammatory cytokines. With most of cytokines unstudied in human diseases, we are optimistic that many new, even better and safer ways of treating autoimmune diseases will emerge.
References

Inflammatory cytokines in autoimmunity


Khader, S. A., Pearl, J. E., Sakamoto, K. et al. 2005. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. J. Immunol. 175:788.


Chan, I. H., Jain, R., Tessmer, M. S. et al. 2014. Interleukin-23 is sufficient to induce rapid de novo gut tumorigenesis, independent of carcinogens, through activation of innate lymphoid cells. Mucosal Immunol. 7:842.


