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Commentary

Cytokines as Therapeutic Targets: Advances and Limitations

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Biological therapies targeting cytokines, T cells, or B cells have improved outcomes of inflammatory diseases. However, many issues remain open: What is the best target? How well can response be predicted? How can cure be achieved?

Introduction

The etiology of immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), psoriatic arthritis (PsA), psoriasis, juvenile inflammatory arthritis (JIA), or inflammatory bowel disease (IBD), remains enigmatic. These disorders can lead to severe organ damage and premature death. On the basis of insights into presumed pathogenetic events, generated over the past two decades, various therapies specifically targeting molecules involved in these pathways have been successfully developed.

RA, a disease that can destroy the joints and is associated with physical disability and enhanced mortality, is likely the best prototypic example for the success of targeted therapeutics, for several reasons. First, it was the first IMID in which such therapy was proven to be efficacious (Elliott et al., 1994). Second, it is the IMID with the largest range of available biological therapeutics (Smolen et al., 2007), and furthermore, many of these drugs are also effective against other IMIDs.

Cells and Cytokines as Pathogenic Culprits and Therapeutic Targets

A variety of cells and cytokines are involved in the pathogenesis of RA [reviewed in (Smolen et al., 2007)]. It is believed that in individuals who are predisposed primarily by carrying a particular major histocompatibility complex (MHC) phenotype, unknown (auto-)antigen(s) are presented by antigen-presenting cells (APCs), such as dendritic cells (DCs), leading to T cell activation. This activation requires additional signaling by costimulatory molecules. Once triggered, T cells activate other cells, especially B cells. These activated B cells then likely produce autoantibodies. T cells also activate macrophages, which secrete a variety of proinflammatory cytokines, including tumor necrosis factor α (TNF), interleukin-1 (IL-1), and IL-6. Autoantibodies, once formed, may form immune complexes that in turn can augment macrophage activation. The proinflammatory cytokines enhance the recruitment, via activation of endothelial cells, of further inflammatory cells into the joint and induce fibroblasts, chondrocytes, and osteoclasts to secrete a variety of inflammatory molecules, including many proteases, which ultimately lead to the swelling and pain, cartilage and bone destruction, and disability typical of RA. They have many activities in common or act in an additive manner, and some may induce others to further improve host defense. Many of these molecules constitute successful therapeutic targets (Figure 1).

Targeted Therapies and Their Proposed Mechanisms of Action

Direct evidence for the pathogenetic involvement of proinflammatory cytokines in RA was provided in the first controlled trial of a monoclonal antibody to TNF (Elliott et al., 1994). Meanwhile, three TNF inhibitors, Etanercept [a construct of TNF receptor (R)2 with an Fc portion of immunoglobulin G], Infliximab (a chimeric monoclonal antibody), and adalimumab (a human monoclonal antibody), are approved for RA, but also for PsA and AS, and an additional two are expected soon (Certolizumab pegol, a pegylated monoclonal Fab' fragment, and Golimumab, a human monoclonal antibody). TNF inhibition is clinically highly efficacious: In combination with a synthetic drug, methotrexate (MTX), a 50% reduction of clinical signs and symptoms is observed in about 50%–70% of treated patients, compared to about 30%–50% on MTX (or biological agent) alone (Smolen et al., 2007). Probably the most impressive aspect of TNF-blocking therapies (plus MTX) is the profound retardation, or often halt, of joint destruction, which even occurs when clinical disease activity continues (Smolen et al., 2005) and can be explained by inhibition of the agonistic effects of TNF on the differentiation and activation of osteoclasts, the cells responsible for the characteristic bone destruction of RA. Interestingly, although all TNF inhibitors are efficacious in several other IMIDs, such as AS, PsA, and psoriasis, only the monoclonal antibodies, not the TNF-R2 construct Etanercept, are able to ameliorate Crohn's disease (Sandborn and Targan, 2002).

Inhibition of IL-1 with the IL-1 receptor antagonist (IL-1Ra) Anakinra (which prevents IL-1 from activating its receptor) is also approved and improves RA, although only 17% of patients (compared to 6% of controls) respond by 50% or more (Cohen et al., 2002). This relatively low efficacy in RA patients is in stark contrast to the profound improvement and even complete reversal of symptoms conveyed by Anakinra in autoinflammatory syndromes including neonatal-onset multisystem inflammatory disease (NOMID) (Goldbach-Mansky et al., 2006) and its efficacy in juvenile- and adult-onset Still's disease. This suggests that, indeed, IL-1Ra is highly effective systemically under circumstances of otherwise potentially lethal, highly febrile disorders. Therefore,

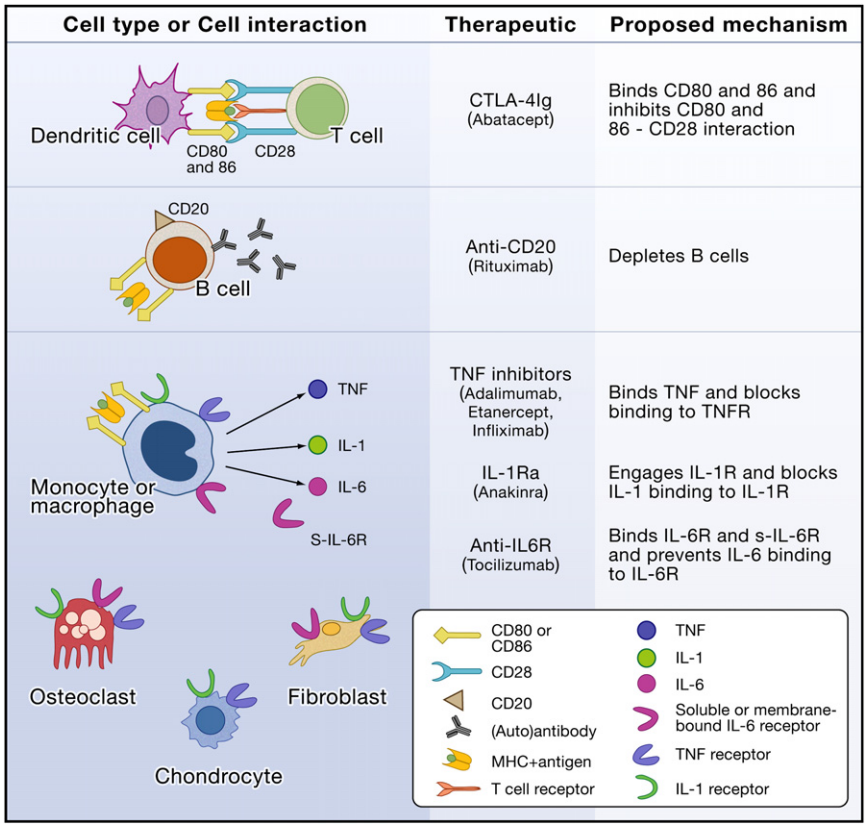

Figure 1. Presumed Pathogenetic Pathways Involved in Rheumatoid Arthritis

For symbols, please see inserted legend.

Top: An antigen-presenting cell (APC), such as a dendritic cell, presents an (auto)antigen via its major histocompatibility complex (MHC)-encoded molecules to the specific T cell receptor. For activation, the T cell requires engagement of its CD28 receptor with costimulatory molecules (CD80 or CD86). Under normal circumstances, cytotoxic T lymphocyte antigen-4 (CTLA-4) is expressed on activated T cells and upon binding to CD80 or 86 downregulates T cell activity (not depicted). Abatacept, a construct of CTLA-4 with an Fc portion of an immunoglobulin G (IgG), binds CD80 and CD86 and thus prevents their engagement with CD28, consequently inhibiting T cell costimulation.

Center: B cells constitute the progeny of (auto)antibody-secreting cells and themselves can serve as APCs by presenting antigen via their MHC and expressing costimulatory molecules such as CD80 and CD86. CD20 is a differentiation antigen of B lymphocytes. Rituximab (anti-CD20) leads to depletion of B lymphocytes, thus eliminating their pathogenetic involvement.

Bottom: Monocytes and macrophages, which can be activated by T cells (but can also serve as APCs and present antigens and costimulatory molecules to T cells), secrete proinflammatory cytokines such as TNF, IL-1, and IL-6, which can engage specific receptors that are expressed on many cell populations (such as monocytes, macrophages, osteoclasts, chondrocytes, and fibroblasts), leading to their activation. TNF inhibitors, such as the human monoclonal antibody adalimumab, the construct of TNF receptor (R)2 with an Fc portion of IgG (Etanercept), or the chimeric monoclonal antibody Infliximab, can bind TNF effectively and thus prevent its engagement with the receptor. The IL-1 receptor antagonist (IL-1Ra, or Anakinra) can engage the IL-1R, thus preventing IL-1 from activating its receptor. Tocilizumab, a humanized antibody to the IL-6R, binds both its membrane and its soluble form and prevents IL-6 engagement and subsequent cell activation via the gp130 chain (not depicted). Effector cells of the final pathogenetic pathways in the joint are osteoclasts leading to bone destruction, chondrocytes leading to cartilage damage, and fibroblasts leading to cartilage damage and propagating synovial inflammation. Please note that all compounds mentioned here are approved for the treatment of RA, except for tocilizumab, which has recently finalized phase III trials and will undergo regulatory authority review in the near future. Certolizumab pegol and Golimumab are additional TNF inhibitors that are not yet approved and will undergo regulatory authority review.

IL-1 plays no important role in RA, it does not sufficiently accumulate in joints, or it has too low avidity to interfere with IL-1 adequately within the joint. However, the fact that IL-1 is induced by TNF in the rheumatoid synovium (Brennan et al., 1989) and that TNF-mediated experimental arthritis acts via IL-1 to a large extent (Zwerina et al., 2007) suggests that there may be a role for IL-1 in RA.

IL-6 is probably the most abundant cytokine present in the joint. IL-6 is induced by TNF and IL-1 and is the major activator of the hepatocytes' acute phase response. Its actual receptor, the IL-6Rα chain, employs an accessory molecule, gp 130, for signal transduction and cell activation. Importantly, gp130 can be activated by both the transmembrane IL-6Rα and its soluble form via trans-signaling (Rose-John et al., 2006). Employment of a humanized antibody to the IL-6Rα chain allows targeting of both the membrane-bound and the soluble IL-6Rα. This antibody, in combination with MTX, appears to convey a similar efficacy as TNF inhibitors, with 50% or more clinical improvement observed in 44% of the patients and 11% of controls (Smolen et al., 2008). Moreover, monotherapy with tocilizumab had good inhibitory effects on progression of joint destruction (Nishimoto et al., 2007). Interestingly, IL-6 inhibition with tocilizumab is also highly efficacious in the treatment of systemic-onset juvenile arthritis (Yokota et al., 2008). Tocilizumab might become approved in Europe in the near future.

Strategies to eliminate B cells with rituximab (anti-CD20) have also been shown to be effective in RA, with about 34% of patients (and 13% controls) attaining at least 50% improvement (Emery et al., 2006), and this is now also a licensed therapy of RA. Dampening of T cell activation with a costimulation inhibitor, abatacept, a construct of cytotoxic T lymphocyte antigen-4 (CTLA-4) fused to an Fc portion of an immunologobulin G molecules (CTLA-4-Ig), is also an effective and approved treatment option for RA. This compound binds to the costimulatory molecules CD80 and CD86 on APC and thus prevents their interaction with their receptor on T cells, CD28, presumably interfering with T cell activation. Abatacept entails at least 50% clinical improvement in 48% of RA patients (controls: 18%) (Kremer et al., 2006).

Challenges of Biological Targeted Therapies

With the advent of biological therapies, the outcome of RA has changed dramatically. Nevertheless, we are far from an optimal situation for a number of reasons.

First, the hierarchy in the course of pathogenetic events is still unresolved. For example, as mentioned before,

targeting of B cells is beneficial, as is targeting of T cell costimulation, TNF, IL-1, or IL-6. But these cytokines activate those cells, and those cells either directly or via other products (autoantibodies, lymphokines) induce the production of these cytokines—therefore, what goes first, the hen or the egg? And if we knew it, would it matter to our therapeutic approach? Would we give one specific treatment before the other one?

Second, with regard to noncytokine-directed, targeted therapies, it is not clear at present how B cell depletion affects RA. Is it by eliminating precursors of autoantibody-producing cells? Is it by eliminating putative antigen-presenting B cells? Is it by eliminating cells that can also contribute to the pool of cytokine producers? Is it necessary to deplete the B cells, or would it be sufficient to interfere with some B cell functions that could be conveyed by a nondepleting B cell-directed therapy? And when it comes to abatacept, is its mode of action solely related to its capacity to inhibit T cell costimulation or to other effects, such as reverse signaling in ligand-expressing antigen-presenting cells, thus, again, potentially inhibiting production of proinflammatory cytokines?

Third, as briefly mentioned above, Etanercept, in contrast to the monoclonal antibodies against TNF, is ineffective in Crohn's disease—why is this the case, given that its effect is comparable in RA, AS, and PsA? One hypothesis suggests that Etanercept may have less apoptotic potential on intestinal T cells as compared to monoclonal antibodies. On the other hand, an anti-TNF Fab' fragment also does not induce apoptosis but still improves Crohn's disease (Nesbitt et al., 2007). Alternatively, Etanercept may affect bacterial lipopolysaccharide-induced IL-1 production to a much lesser extent than anti-TNF (Nesbitt et al., 2007), although it is not clear whether this is the reason for the differential therapeutic effects in Crohn's disase. A further notion in this respect relates to the observation that TNF-R2 is upregulated in Crohn's disease and that its overexpression in experimental systems promotes bowel inflammation (Holtmann et al., 2002). One might speculate that the intestinal TNF-R2 molecules, once overexpressed, attract more local TNF than can be bound by the circulating TNF-R2 construct.

Another interesting observation relates to the often good efficacy of a second TNF inhibitor once the initial TNF-inhibitor has failed to convey therapeutic benefit (Hyrich et al., 2007). Of course, this may have pharmacogenomic reasons, but it is a riddle nevertheless and shows the complexity of therapeutic decision making even with similar targeted therapies.

Why Do We Fail to See Additive Effects of Targeted Therapies?

All biological therapies currently applied have better efficacy if combined with synthetic drugs used in RA, such as methotrexate. What are the mechanisms leading to this increase in benefit compared to monotherapy with biological agents? On the other hand, one would postulate that targeting several specific molecules involved in pathways leading to the disease with two (or more) biologicals would have added benefit. Alas, neither combinations of inhibitors of different cytokines nor of different pathways (Weinblatt et al., 2006) have demonstrated clinical advantages so far. The adverse events, however, especially the rates of infections, were increased, suggesting that the combination led to the inhibition of both targets, albeit without a beneficial effect on the inflammatory response in RA.

On the other hand, denosumab, an antibody directed to receptor activator of nuclear factor kappa B ligand (RANKL), a pivotal molecule for the differentiation and activation of osteoclasts, may specifically inhibit osteoclast activation and bone destruction and thus could be a sound candidate for combination therapy with agents that have good effects on symptoms but lesser effects on bone damage. However, this antibody does not appear to halt progression of cartilage damage (van der Heijde et al., 2007). Thus, the question arises whether the mere inhibition of bone but not cartilage damage will be sufficient to interfere with long-term disability in RA.

What Are the Best Ways of Targeting the Effects of a Cytokine?

It was previously discussed that Anakinra (L-1Ra) appears to have only little efficacy in RA and that this could be due to a combination of a compound with relatively low avidity to the receptor and a disease involving a distant region, the joint, and that the effects of antibodies targeting IL-1 remain to be seen. A further open question is also whether it is better to target the IL-6R than to target just IL-6.

Also with respect to TNF inhibition there are a variety of open issues. TNF signals via at least two receptors, the p55 and the p75 receptors, which have different though partly overlapping effects (Holtmann and Neurath, 2004). As mentioned above, TNF-R2 (but not TNF-R1) appears to be overexpressed in Crohn's disease. In experimental arthritis of mice overexpressing soluble TNF, TNF-R1 appears to be driving inflammation and especially osteoclast-mediated destruction. Interestingly, the absence of TNF-R2 led to an increase not only of synovial inflammation but also joint destruction, indicating a dual mechanism of TNF (Blueml et al., 2007). Thus, rather than targeting TNF, it may be worthwhile to target different TNF receptors in different diseases (Holtmann and Neurath, 2004), leaving the other TNF-signaling pathway intact and thereby possibly increasing safety.

When Will We Be Able to Individualize Therapy?

It is currently not possible to predict which patients will respond to what extent to which type of treatment. Aside from synthetic agents, whose mode of action has not been ultimately revealed, we have therapies available that specifically target costimulation, B cells, TNF, IL-1, and, in the future, IL-6. These therapies are effective in many patients—but still we have no clue in whom these treatments will work well and whether the patients with very good responses are distinct populations for each of these or constitute overlapping groups. Currently available biomarkers, with the exception of acute-phase reactants, have little predictive capacity with regard to clinical outcomes or joint damage.

How Good Are We Really?

Currently, we can achieve stringent remissions, i.e., no evidence of active disease, with consequential therapy in about 20% of the patients in clinical practice. However, cure is not yet in sight. Although cure will ultimately require knowing the cause or causes of these disorders, it is conceivable that interference with the vicious cycle of the inflammatory occurrences very early in the course of the disease process may reverse the events usually destined to become chronic in

predisposed individuals. Such window of opportunity is addressed by currently ongoing clinical trials, and it remains to be seen if this hypothesis can come true.

And What About the Risks of Targeted Therapeutics?

In these types of therapies, there is rarely a benefit without potential harm. All compounds above block cellular and/or molecular functions that presumably have an important role in the healthy host. Foremost, TNF, IL-1, and IL-6 play important roles in host defense both in the innate and adaptive immune systems. Therefore, one should expect an increase in the rates of infections, which is the case (Smolen et al., 2007; Cohen et al., 2002; Emery et al., 2006; Kremer et al., 2006; Smolen et al., 2008). For example, TNF plays a pivotal role in granuloma formation and, therefore, in the defense against intracellular pathogens. Indeed, reactivation of tuberculosis has been observed with TNF inhibitors. In contrast, IL-6 may have an inhibiting role on granuloma formation (Nagabhushanam et al., 2003); therefore, targeting IL-6 therapeutically might not induce reactivation of, or promote infections with, some intracellular pathogens.

Other aspects relate to increases in lipid levels in the course of IL-6 inhibition. It remains to be seen whether cardiovascular risk increases. Presently, rather the reverse has been observed in patients in whom inflammatory disease was successfully treated and lipids increased as a consequence of the anti-inflammatory treatment effects. IL-6 is also a growth factor for hepatocytes, and tocilizumab leads to increases in hepatic enzymes, which are usually of transient nature and not associated with hepatitis (Smolen et al., 2008). However, long-term follow up will have to show whether this might be associated with liver damage. Given that TNF inhibition also leads to normalization of IL-6 (Charles et al., 1999) but not to liver enzyme elevations, it will also be of interest to learn whether a monoclonal anti-IL-6, rather than an antibody to the IL-6R, will induce a similar adverse event profile.

Some of these biological agents are chimeric or humanized monoclonal antibodies. Therefore, sensitization might occur, which can lead to both allergic reactions and reduction in efficacy.

Future Directions

Finally, many additional molecules are potential targets for future effective therapies. These comprise other cytokines than those currently aimed at and signal-transduction molecules. For example, inhibition of Jak3, in early studies, has shown an interesting efficacy profile (Breedveld et al., 2007).

How can one learn more about early effects of such agents? One way might be to employ in vivo microscopy, which has successfully been used already elsewhere (Castellino et al., 2006), in experimental models of arthritis in the course of application of these treatments. This could allow some of the tantalizing questions to be answered: When different molecules or cells are targeted, will the composition of the synovial inflammation change dependent on the compound employed? Or, do all T cells present in arthritic joints of animals with antigen-mediated arthritis have antigen specific properties? Or, what are the earliest immunologic events within the joint in different forms of experimental arthritis, and how do they react to different treatment modalities? Although the answers may not necessarily translate fully to the human situation, they may at least give a clue on which cells may be initially affected and what these effects mean in the context of the cellular composition of the synovial membrane and the cytokine profile expressed. Another important aspect relates to prospects to predict severity of disease and response to therapy. It is here where there is hope for deeper insights to be gained by genomic and proteomic analyses—but this will also have to await better-designed comparative clinical trials that allow the respective questions to be asked.

In summary, targeting of proinflammatory cytokines such as TNF or IL-6 is highly efficacious in rheumatoid arthritis and, at least for anti-TNF agents, also other chronic inflammatory rheumatic and non-rheumatic inflammatory disorders. They substantially improve signs and symptoms and retard or prevent organ damage and disability, the most devastating consequence of these chronic conditions. Although the progress made over the past dozen or so years has dramatically improved the fate of the patients, we still lack sufficient predictive insights to determine the optimal therapeutic strategy for the individual patient. Moreover, despite all these advances, the overall rate of good responses is limited, with only about 10%–40% of patients achieving improvement of 70% or more with any one of these agents based on clinical scoring. Thus, although with increasing therapeutic options an increasing number of patients will achieve a good clinical result and, ideally, remission, new compounds with even better efficacy and better safety will still be needed. In parallel, the search for causes, the search for predictors, and the search for explanations of the effects of many of these therapies will have to go on.

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