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An Overview of Cytokines and Cytokine Antagonists as Therapeutic Agents

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Abstract

Cytokine-based therapies have the potential to provide novel treatments for cancer, autoimmune diseases, and many types of infectious disease. However, to date, the full clinical potential of cytokines as drugs has been limited by a number of factors. To discuss these limitations and explore ways to overcome them, the FDA partnered with the New York Academy of Sciences in March 2009 to host a two-day forum to discuss more effective ways to harness the clinical potential of cytokines and cytokine antagonists as therapeutic agents. The first day was focused primarily on the use of recombinant cytokines as therapeutic agents for treatment of human diseases. The second day focused largely on the use of cytokine antagonists as therapeutic agents for treatment of human diseases. This issue of the *Annals* includes more than a dozen papers that summarize much of the information that was presented during this very informative two-day conference.

Keywords

cytokines; inflammation; interferons; interleukins; receptors

Introduction

Cytokine therapies have great potential for treating a variety of diseases. These intercellular messengers activate numerous signaling pathways in virtually all cell types, but they are perhaps best known for their role in recruiting and activating various types of leukocytes in response to injury or infection. The actions of cytokines can be either positive or negative as concerns clinical context. For example, proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) often contribute to the pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), but appear to be essential for salutary responses to infectious agents. Similarly,

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Conflicts of interest

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cytokines can also enhance or inhibit the development of many cancers via their effects on pathways promoting tumorigenesis and tumor metastasis.

The therapeutic potential of cytokines was recognized decades ago, but attempts to use recombinant cytokines as conventional drugs have been mixed. Interleukin-10 (IL-10) is one example of a cytokine in which preclinical studies suggested great potential,¹ but therapeutic effectiveness was not demonstrated when tested in controlled human clinical trials.² After the clinical trials conducted in the 1990s failed to demonstrate significant improvement in patients with IBD, this cytokine was largely dropped from further clinical development. The unsuccessful use of cytokines, such as IL-10 and IL-12, as therapeutic agents was likely assured by using them like conventional drugs in terms of dosage and route of administration. Because cytokines have activity on many different cell types and tissues, the serious adverse events associated with IL-12 therapy and the lack of efficacy associated with both IL-12 and IL-10 may well be attributable to the systemic rather than more localized administration of these cytokines.

Many of the early clinical trials of cytokines conducted in the 1990s, including the IL-10 and IL-12 trials, were conducted without a full understanding of cytokine function and regulation. As endogenous proteins, cytokines are regulated by various homeostatic mechanisms that can modulate their activities as therapeutic agents. They are also subject to biological degradation, and thus most have very short half-lives *in vivo*.

As discussed during this conference, scientists are now developing more effective methods to administer cytokines and to combine them with other treatments to maximize their effectiveness while minimizing their toxicities. They have developed novel ways to prolong the biological activity of these molecules and to deliver them in a more targeted manner to enhance efficacy and minimize toxicity. Furthermore, many new cytokines have been discovered since the time when the clinical trials of recombinant human IL-10 and IL-12 were conducted. Consequently, the number of defined cytokines has increased significantly as a result of advances in genomic database-mining techniques. For example, there are now 35 interleukins as well as several novel interferons (IFNs). Many of these more recently identified cytokines will be tested clinically in the near future, so the important lessons to be learned from the earlier clinical experiences with such cytokines as IL-2, IL-10, and IL-12 must be brought to bear with respect to development of these novel cytokines.

Many cytokines such as IL-1 and TNF- α are inherently toxic because of their marked proinflammatory activities. Thus these cytokines have proved excellent targets for development of antagonists that block their production or activity. Consequently, many highly effective cytokine inhibitors have been developed during the last decade, including some very effective and highly selective inhibitors of IL-1 and TNF- α . It is possible, and perhaps likely, that many of the more recently discovered cytokines, such as IL-22 and IL-23, will also prove useful therapeutic targets for development of specific antagonists.

Gaining a more complete understanding of cytokine networks and developing more selective delivery methods are only two parts of the current challenge regarding these molecules. A third part is to convince academic, pharmaceutical, and regulatory scientists to agree on a

common path forward that will maximize the possibility of clinical success of novel cytokine therapies. For example, regulatory requirements and intellectual property issues should not impede development and clinical testing of potentially life-saving therapies for patients with unmet medical needs. The FDA's Critical Path Initiative (<http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative>) provides a useful platform to help bring these distinct stakeholders together to discuss how to rejuvenate efforts to test promising cytokines, as well as to shorten the lag between initial drug discovery and regulatory approval.

To discuss current topics regarding the clinical use of cytokines and cytokine antagonists, scientists and clinicians from academia, the biopharmaceutical industry, and FDA met together at the New York Academy of Sciences on March 26–27, 2009. The goal of this two-day conference was to provide a forum to review lessons learned from previous clinical trials of many cytokines and cytokine antagonists and to consider novel approaches to improve the effectiveness of cytokines as therapeutic agents in future clinical trials. The program was co-organized by Drs. Raymond Donnelly and Amy Rosenberg of the FDA (Bethesda, MD) and Howard Young of the National Cancer Institute (Frederick, MD).

Day 1: Cytokines as Therapeutic Agents

Interferons as Agents for Treating Infectious Diseases

Cytokines play an important role in the host response to microbial pathogens, such as bacteria and viruses. The interaction of microbial pathogens with cell surface receptors, such as the *Toll*-like receptors (TLRs) or the entry of viruses into a cell often activates transcription of many cytokine genes and production of the corresponding proteins. These cytokines are often secreted by infected cells and deliver secondary warning signals to nearby cells to activate their innate antimicrobial defenses. Cytokines can also act on many of the cell types that constitute the host immune system, including B cells, T cells, natural killer (NK) cells, and macrophages. One such antiviral cellular response occurs when the cytokine IFN- α binds to its receptors. IFN- α receptors are expressed on virtually all somatic cell types, and binding of IFN- α to its cognate receptor complex induces expression of many so-called interferon-stimulated genes (ISGs). Many of the protein products encoded by these genes then act to induce antiviral activity in surrounding cells.

IFNs are a group of cytokines that can induce potent antiviral activity in many cell types. Type-I IFNs include IFN- α , IFN- β , and IFN- ω , all of which bind and signal through a common IFN- α receptor complex. There are thirteen types of human IFN- α , one type of IFN- β , and one type of IFN- ω . There is only one type-II IFN, known as IFN- γ , which binds and signals via the distinct IFN- γ receptor complex (Fig. 1). More recently, a novel group of IFNs was discovered that makes up a third family of IFNs now known as the type-III IFNs.^{3,4} This new group of IFNs includes three members: IFN- λ 1, - λ 2, and - λ 3. These cytokines bind to a distinct receptor denoted IFN- λ R1 (also known as IL-28RA).^{3,4}

IFN- α is an approved treatment for chronic hepatitis C virus (HCV) infection. If left untreated, HCV infection can progress to liver cirrhosis and eventually cancer (hepatoma) in a subset of patients. The current standard of care for the treatment of chronic HCV infection

is to administer a pegylated form of IFN- α in combination with the antiviral drug ribavirin. Addition of the polyethylene glycol (PEG) moiety to IFN- α prolongs the half-life of the protein in the body. Unfortunately, this treatment regimen (pegylated IFN- α plus ribavirin) significantly reduces viral load in only about 50% of patients who are infected with HCV genotype-1. For additional information, please see: <http://digestive.niddk.nih.gov/ddiseases/pubs/chronichepc/index.htm>.

One approach to improving the efficacy of hepatitis C treatment regimens is to increase the overall bioactivity of IFN- α . Each of the naturally occurring IFN- α molecules has a slightly different efficacy profile, suggesting that a chimeric protein might be even better than the native isoforms. Currently, there is one FDA-approved IFN- α product, known as Infergen, that was derived from a consensus sequence of the 12 naturally occurring human IFN- α proteins. There are also two versions of recombinant human IFN- α 2, known as Roferon and Intron A, available for clinical use in the United States.

Dr. Julian Symons of Roche-Palo Alto, LLC, and his colleagues investigated whether a novel technology known as “gene shuffling” could be used to increase the ability of IFN- α to stimulate kill of viruses and modulate the immune response.⁵ Libraries of gene-shuffled IFN molecules were tested for their antiviral, immunomodulatory, and antiproliferative activities. After several rounds of selection, they chose the candidate that had the strongest antiviral activity. This designer IFN protein demonstrated enhanced antiviral activity *in vitro* when compared to other IFN- α proteins. Consequently, this hybrid IFN was tested in a small clinical trial in patients with chronic HCV infection. Unfortunately, this protein induced production of anti-IFN- α antibodies in many patients, and these antibodies neutralized the biological activity of the drug. Further development of this cytokine as a novel drug candidate was halted. The development of anti-IFN antibodies in patients who were treated with this hybrid IFN molecule may have been caused by the fact that this protein contained novel, non-native stretches of amino acids that were perceived as foreign by the host immune system. Such responses may extend to conserved regions of the molecule and produce neutralizing responses in a process called *epitope spreading*. This clinical experience underscores the profound impact that even a few amino acid differences can impart on the relative immunogenicity of a protein.

Conventional antiviral therapy with IFN- α products, such as Pegasys (peginterferon α -2a) or PegIntron (peginterferon α -2b) often induces many undesirable side effects, including headache, fevers, chills, nausea, and myelosuppression. Consequently, scientists are seeking alternative, less toxic agents to treat this disease. One promising, novel, antiviral agent that was discussed by Dr. Dennis Miller of ZymoGenetics, Inc. (Seattle, WA) is IFN- λ . IFN- λ , also known as IL-29, is functionally similar to IFN- α but is potentially much less toxic than IFN- α as a treatment for chronic HCV infection.⁶

Both IFN- α and IFN- λ are induced by viruses *in vitro* and *in vivo*, but they bind to distinct receptors (Fig. 1). The IFN- λ receptors are expressed on fewer cell types than are those for IFN- α . However and critically, like IFN- α receptors, IFN- λ receptors are expressed on liver cells, the primary cellular targets of the HCV. The fact that expression of IFN- λ receptors is much more restricted than IFN- α receptors suggests the possibility that IFN- λ will induce

fewer side effects than IFN- α . In terms of receptor binding kinetics, IFN- α binds and releases from its receptor relatively quickly, whereas IFN- λ binds and releases from its receptor more slowly. Both cytokines induce expression of the same subset of ISGs and appear to share common antiviral, antiproliferative, and apoptosis-inducing activities.^{7,8}

In a Phase-1 clinical trial, ZymoGenetics's pegylated version of IFN- λ 1 (IL-29) was well tolerated and did not induce the neutropenia, thrombocytopenia, or anemia that commonly occur in patients who are treated with IFN- α products. Several patients in their initial Phase-1 clinical trial demonstrated significant decreases in HCV RNA levels, and the sponsor plans to continue studying this novel drug in a Phase-2 clinical trial.⁶

Cytokines, particularly the IFNs, have the potential to treat many other infectious diseases in addition to hepatitis C. For example, as discussed by Dr. Howard Young and his colleagues at the National Cancer Institute (Frederick, MD), IFN- γ has been shown to be a very effective agent for treating several types of infectious disease, including drug-resistant tuberculosis.⁹ However, this cytokine activates many inflammatory responses throughout the body and induced an unacceptable level of adverse effects in these trials. Dr. Steven Holland of the National Institute of Allergy and Infectious Diseases (NIAID) pointed out that, after many unsuccessful clinical trials for other indications, because of toxicity, IFN- γ was eventually approved by the FDA only as a treatment for two, rare genetically inherited conditions: chronic granulomatous disease (CGD) and osteopetrosis.

Despite these limited clinical applications, continued exploration of the role of endogenous IFN- γ in the physiologic response to infectious agents has led to new treatment options for people with specific inherited defects in the IFN- γ receptor gene.¹⁰ For example, a very small number of people do not express IFN- γ receptors and as a result are unable to mount an effective immune response against certain microbial pathogens, including some types of mycobacteria. Holland and his team successfully treated one such patient suffering from disseminated *Mycobacterium avium* by treating her with IFN- α .¹¹ However, they also found that IFN- α cannot substitute for IFN- γ in all types of infections. A greater understanding of cytokine-induced signaling pathways might help clinicians devise more specific treatments for various diseases.

Interleukin-7 as an Immune System Rejuvenator

The study of cytokine mechanisms can reveal not only treatments for specific diseases but also ones with broad potential to rejuvenate or reinforce the immune system. "One cytokine with broad immunostimulatory potential is IL-7," said Dr. Crystal Mackall of the Pediatric Oncology Branch of the National Cancer Institute. IL-7 is a member of a broader subset of cytokines that also includes IL-2, IL-4, IL-9, IL-15, and IL-21. Each of these cytokines binds initially to its unique ligand-binding (alpha) chain. However, they all share the use of the IL-2 receptor common gamma chain (γ c) as a secondary receptor component that is essential for signaling.

According to Dr. Mackall, IL-7 can restore certain T-cell populations that are diminished naturally over time. Normally, these T cells are replenished in two ways, either via a thymic-

dependent process or via a thymic-independent process called homeostatic peripheral expansion (HPE).¹²

IL-7 predominantly promotes the regeneration of T cells via the non-thymic-dependent process. Thus, IL-7 could potentially be of benefit to patients who have undergone thymic involution, including the elderly, since as people age, the thymus becomes less efficient as a source of T-cell generation. A non-thymus-dependent method of generating T cells might also increase the levels of such cells in patients in whom T cells have been depleted by treatments, such as those undergoing chemotherapy and bone marrow transplantation, or by infection such as those with HIV infection. IL-7 preferentially expands naive T cells and increases overall T-cell repertoire diversity. No serious adverse effects were observed during the initial human clinical trials of IL-7.¹²

Cytokines as Anticancer Agents

A Fresh Look at IL-2—The use of cytokines, such IL-2, as a treatment for certain types of cancer results in complete remission in a very small subset of patients and a significant but less than full response in a small number as well. It is clear that more research needs to be done in order to learn how to extend this limited clinical success to a larger number of patients.¹³

The antiproliferative properties of cytokines are mediated via a number of mechanisms. They can induce direct antiangiogenic effects or indirectly induce expression of genes that are antiproliferative. Cytokines, in general, and IL-2 in particular, can also induce apoptosis, a type of programmed cell death, either directly by activation-induced cell death, requiring expression of the death receptors Fas and Fas ligand, or indirectly via activation of cytotoxic T lymphocytes that kill cells via an apoptotic mechanism.

“In the early 1990s, IL-2 raised great hopes of providing a treatment or perhaps even a cure for some types of cancer,” said Dr. Michael Lotze of the University of Pittsburgh Cancer Research Institute. Treatment with this cytokine induces a durable remission rate in a small subset of patients (8–10%) with melanoma and renal cell carcinoma.¹³

However, the small percentage of patients in which success is demonstrated is not sufficiently robust. Therefore, work was focused on defining the mechanism by which this therapy works in that small subset of responders. Also, given the severe toxicities associated with IL-2 treatment, studies have also focused on profiling patients that might truly benefit from the treatment. A recent study by Howard Kaufman’s lab examined responsiveness and non-responsiveness to IL-2 therapy and found that individuals with high levels of the growth factor, VEGF, prior to therapy were less likely to respond compared to patients with low concentrations of VEGF in their bloodstream.¹⁴ Similarly, fibronectin was high in nonresponders but low in responders. These levels could perhaps be used to prospectively identify patients who will respond to IL-2.

Another way to understand the low rate of success with IL-2 therapy is to examine its activity in the context of the cell’s response to certain growth factors and stress. “Cancer can be considered to be a metabolic disorder because the body’s metabolism supports tumor

formation,” said Lotze. The immune system is complicit because it tolerates formation of the tumor and surrounding new blood vessels. Scientists have known for some time that apoptosis is disabled during tumor formation. Lotze believes that a competing cell death process called *autophagy*, in which cells catabolize their own cellular components when cells are under survival stress, allows many tumor cells to survive anticancer treatments. He further noted that VEGF and HMGB1 are important switches that modulate apoptosis and autophagy.¹³ This would suggest that the high levels of VEGF associated with poor response to IL-2 may interfere with apoptosis by favoring autophagy and would further suggest use of monoclonal antibodies (mAbs) to VEGF as a mechanism to facilitate beneficial effects of IL-2 in patients with high VEGF levels.

To improve the remission rate of IL-2, we need to acquire a better understanding of the spectrum of IL-2 effects on cellular and soluble mechanisms that affect survival and death of tumor cells. In addition to induction of apoptosis, IL-2 directly activates NK cells and macrophages, promotes Th1 cell activity, and induces proliferation of B cells. IL-2 therapy for cancer is also associated with induction of autoimmunity, which, in the setting of IFN- α treatment, appears to correlate with clinical benefit.

Defining the Mechanisms by Which Cytokines Mediate Their Activity—Dr.

Ahmad Tarhini of the University of Pittsburgh Cancer Institute described the clinical and immunological basis of IFN- α therapy in melanoma patients.¹⁵ Melanoma is a highly curable cancer if diagnosed and treated aggressively in the early stages of development but is usually fatal if allowed to metastasize. Some spontaneous regressions occur, often in the setting of autoimmune phenomena, suggesting that the immune system can suppress this disease, perhaps through the involvement of T cells, macrophages, and NK cells.

Tarhini and his colleagues are attempting to identify biomarkers that may help to predict which patients will respond favorably to IFN- α treatment. They have found that patients with high pretreatment levels of certain proinflammatory cytokines, such as IL-1 α , MIP-1 α , IL-6, and TNF- α are more likely to have relapse-free survival. They also observed that survival of melanoma patients following IFN- α treatment was greater in patients who developed autoimmunity such as vitiligo or thyroiditis.¹⁵ These data speak to autoimmunity or a high potential for its generation, as a means to effectively target and kill tumor cells. Indeed, the presence of T-cell infiltrates in the tumor is a positive prognostic marker, and the presence of T-cell infiltrates within regional nodal metastases often predicts positive responsiveness to IFN- α treatment.^{16,17}

Dr. Ernie Borden of the Cleveland Clinic underscored the need to understand the intracellular mechanisms that occur downstream of IFN binding to its cell-surface receptor. Borden indicated that, to fully exploit the therapeutic potential of IFNs, we must gain a better understanding of the regulation and function of the more than 300 ISGs that are induced by this cytokine. Some of these genes, such as TRAIL and XAF1, are proapoptotic, whereas other genes such as G1P3 (ISG 6–16) inhibit apoptosis. Many other IFN-inducible genes are immunomodulatory or antiangiogenic. Defining the precise function(s) of ISGs may help scientists to overcome the resistance mechanisms and drug-related toxicities that

are associated with IFN- α therapy. Such studies may also reveal improved ways to enhance the antitumor activity of the IFNs.

One common downstream signaling pathway that is activated by IFN- α is the JAK/STAT pathway. A drug known as stibogluconate (SSG) enhances activation of the JAK/STAT pathway by IFN- α and thereby increases the magnitude of activity induced by IFN- α . The combination of stibogluconate and IFN- α is now being tested in a Phase 1 clinical trial in melanoma patients.¹⁸

IL-21 in Addition to Monoclonal Antibodies for the Treatment of Cancer—IL-21, a relative newcomer in the field of cytokines, is also being tested as an anticancer therapeutic agent.¹⁹ This cytokine is secreted by activated CD4⁺ T cells and NK cells. It helps regulate immunoglobulin production and Ig-isotype switching by B cells and has activating effects on macrophages. IL-21 has demonstrated anticancer properties in tumor-modeling studies in mice as well as in early clinical trials in humans. In his presentation, Dr. William Carson (Ohio State University) pointed out that IL-21 has structural homology to other class-I cytokines, including IL-2. His group has shown that this cytokine can induce antitumor responses in murine models of melanoma, renal cell carcinoma, colon adenocarcinoma, breast cancer, and other tumors. The antitumor effects of IL-21 appear to be mediated largely by NK cells and CD8⁺ T cells.¹⁹

Dr. Carson and his colleagues evaluated the antitumor activity of recombinant IL-21 in combination with trastuzumab (Herceptin, Genentech, Inc.), a monoclonal antibody that inhibits the growth of Her2/Neu-positive tumors and mediates antibody-dependent cellular cytotoxicity (ADCC). They found that IL-21 enhances NK cell-mediated ADCC and cytokine production when administered in combination with several different monoclonal antibodies, such as rituximab (Rituxan, Genentech) or trastuzumab. These findings suggest that combined treatment with the cytokine IL-21 plus an appropriate monoclonal antibody may yield more robust and sustained antitumor responses than can be achieved by monotherapy with either agent alone.¹⁹

Lessons Learned

Since the time when many of the early, cytokine-based clinical trials were first conducted (1990–2000), numerous technological advances have occurred that can revive the clinical potential of at least some of these cytokines as drug candidates. These include advances in drug delivery platforms and methods to prolong the *in vivo* half-life of therapeutic proteins. Additionally, biomarkers can now be used to prospectively identify patients who would be more likely to respond to a particular cytokine. Many of the new analytical tools that exist today, particularly microarray technologies, can also be used to identify better biomarkers of cytokine-mediated pharmacodynamic activities.

Some of the cytokines that failed in clinical trials in the 1990s might now be worth reevaluating. A good example of one such cytokine is IL-12, which was largely abandoned after the original clinical studies of this cytokine were conducted in the 1990s. This cytokine was evaluated as a potential treatment for certain types of cancer and infectious disease but was found to be highly toxic and largely ineffective as a mono-therapeutic agent in several

clinical trials. However, it is now clear that IL-12 might be more effective as an anticancer agent if administered at lower, less toxic concentrations together with other anticancer drugs or cytokines.

Day 2: Cytokines as Therapeutic Targets

Inflammatory autoimmune diseases such as RA and psoriasis can often be treated successfully with cytokine antagonists, such as the TNF inhibitors, Enbrel, or Remicaide. These biological agents may also be useful for treating other clinical indications for which there are currently no alternative treatment options. In addition, many novel cytokines have been discovered during the last ten years, and it is reasonable to predict that at least some of these cytokines will provide useful therapeutic targets for development of novel cytokine antagonists.²⁰

Cytokines and Cytokine Antagonists for the Treatment of Autoimmune and Inflammatory Diseases

Key discussion points:

- Proinflammatory cytokines induce many of the pathogenic processes that are characteristically associated with many autoimmune diseases.
- Monoclonal antibodies that block the activity of specific cytokines, either by blocking cytokine receptors or neutralizing cytokine activity, are often highly effective for the treatment of such autoimmune diseases as RA, IBD, and psoriasis.
- Gene expression profiling may help scientists to better understand how cytokines regulate the inflammatory processes that are associated with many autoimmune diseases.
- New methods for targeting and delivering cytokines and cytokine antagonists have the potential to increase the therapeutic utility of these agents and reduce their undesirable toxicities.

Cytokines often function as intercellular messengers to activate the immune system, and they play a central role in many diseases that involve the immune system. These diseases include inflammatory diseases characterized by excessive production of inflammatory cytokines, such as TNF- α and/or IL-1, and autoimmune diseases that are characterized by immune responses directed against the body's own proteins or cells. Effective treatment of many immune-mediated disorders has been improved greatly by the discovery of cytokine inhibitors. These include cytokine receptor constructs that can bind and neutralize specific cytokines and monoclonal antibodies that target specific cytokines.

RA is a classic example of an inflammatory disease where cytokines play a prominent role. RA is characterized in part by the infiltration and activation of inflammatory T cells that produce proinflammatory cytokines, such as TNF- α , IL-1, and IL-6. These cytokines, in turn, mediate the activation of tissue-destroying metalloproteinases and expression of vascular adhesion molecules that recruit lymphocytes, macrophages, and other types of

leukocytes to the joints. As a consequence of these events, B cells often become activated and produce autoantibodies. If not blocked pharmaceutically, these processes can lead to progressive joint destruction.

Rheumatoid Arthritis

For many patients, RA can also affect organ systems other than the joints. For example, patients can also develop cardiovascular disease, chronic pulmonary obstructive disease (COPD), blood disorders, neurological symptoms, pulmonary effects, and ocular problems. Dr. Larry Moreland of the University of Pittsburgh reviewed ways to specifically target TNF- α , a proinflammatory cytokine that plays a central role in the pathogenesis of RA.²¹

TNF- α appears to play a central role in disease activity in roughly three-quarters of all RA patients. Currently, five biologic agents that inhibit TNF- α are approved for clinical use in the United States. Three are mAbs against TNF- α , and two are soluble receptor constructs that act by binding TNF- α and facilitating its clearance from the body. The TNF inhibitors such as Enbrel and Remicaide have been shown to decrease symptoms, slow disease progression, and improve the quality of life for many patients with RA.²¹

It is unclear how frequently patients develop neutralizing antibodies to TNF antagonists such as Enbrel or Remicaide after being treated with one or more of these drugs for some period of time. Comparative studies to accurately quantify the incidence of anti-TNF antibodies in RA patients treated with different anti-TNF agents have not yet been reported. Furthermore, TNF- α may not be the most appropriate cytokine to target in all RA patients. Other cytokines, such as IL-1, IL-6, or IL-12 may be the dominant disease-driving cytokine in RA patients who do not respond well to TNF inhibitors. The use of contemporary cytokine-profiling platforms could provide very useful information regarding which patients will respond most favorably to specific anticytokine therapies.

Blocking IL-12 and IL-23 as a Therapy for Autoimmune Diseases

Psoriasis is an inflammatory disease that is characterized in part by the rapid growth of skin cells. Scientists have found that in classic psoriasis T cells often become activated to produce multiple cytokines that stimulate the growth of keratinocytes. One such cytokine is IL-12, which acts on Th1 cells to induce production of IFN- γ . Another highly related cytokine, IL-23, acts preferentially on Th17 cells to stimulate production of a distinct subset of proinflammatory cytokines, including IL-6, IL-17, and IL-22.

Michael Elliott of Centocor, Inc. discussed clinical development of ustekinumab (Stelara), a humanized mAb that binds the shared p40 subunit of IL-12 and IL-23.²² This mAb binds with high affinity to the p40 subunit of IL-12 and IL-23 and prevents these cytokines from binding to the IL-12R β 1 receptor. This in turn prevents activation of the intracellular signaling cascade that is normally activated by these cytokines. This novel cytokine antagonist is approved for marketing in Canada and Europe for moderate to severe plaque psoriasis. It has also recently been approved by the FDA for use in the United States.

By blocking both IL-12 and IL-23, ustekinumab inhibits inflammatory cell infiltration, decreases expression of such cytokines as IFN- γ , IL-17, and IL-22, and reduces epidermal

hyperplasia. This drug does not appear to affect the levels of circulating Th1, Th2, Treg, or NK cells, although there may be some reduction in the levels of circulating Th17 cells. In addition to its use as a treatment for chronic psoriasis, ustekinumab may also be useful as a treatment for other autoimmune diseases such as psoriatic arthritis and Crohn's disease.²²

Interferon- β in Autoimmune Disease

Richard Ransohoff of the Cleveland Clinic Foundation pointed out that recombinant human IFN- β is very effective as a treatment for multiple sclerosis (MS) in at least a subset of patients.²³ However, treatment with recombinant human IFN- β is not uniformly effective in all MS patients, and it has several negative features. It is expensive, inconvenient, and induces many undesirable side effects. Nevertheless, prior to its approval by the FDA for clinical use as a treatment for MS, there were very few treatment options for patients with this disease.

Scientists would like to be able to predict which patients will respond positively to IFN- β therapy. To identify predictive biomarkers, Ransohoff and his colleagues evaluated gene expression profiles from multiple MS patients to see if individuals treated with IFN- β had varied responses to this cytokine. They used microarrays containing a large subset of ISGs to examine changes in gene expression levels following IFN- β treatment.²³

They found that neither the magnitude nor the stability of the biological response to IFN- β was responsible for differences in responsiveness among patients. They concluded that a specific gene or group of genes that are induced by IFN- β must account for the differential responsiveness of patients to IFN- β therapy. Their group and others have recently identified a number of genes that are commonly associated with MS, and they are currently exploring how these genes are regulated by IFN- β treatment.²³

Another team that is using gene expression profiling to learn more about how cytokines influence diseases is headed by Dr. Virginia Pascual of the Baylor Institute for Immunology Research. Pascual and her team are looking for gene signatures associated with systemic lupus erythematosus (SLE). They have determined that ISG signatures provide useful biomarkers for diagnosis and assessment of disease activity in SLE.²⁴

IL-1 in Autoinflammatory Disease

Research in rare genetic diseases can help expand our understanding of inflammatory processes in more common diseases, explained Dr. Raphaela Goldbach-Mansky of the Translational Autoinflammatory Disease Section at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).²⁵ Three such rare diseases are collectively called cryopyrin-associated periodic syndromes (CAPS). Familial cold autoinflammatory syndrome (FCAS) involves cold-induced attacks of fever, neutrophilic urticaria, conjunctivitis, and joint pain, lasting 12 to 24 hours and then resolving. Muckle Wells Syndrome (MWS) is a more severe and persistent disease that is not cold induced and involves fever, neutrophilic urticaria, joint pain, progressive hearing loss, and amyloidosis. The third disorder is neonatal onset multisystem inflammatory disease (NO-MID), involving the same symptoms as MWS, but including bony overgrowth of the knees, organ damage, and mental retardation.

All three of these diseases appear to be mediated by the proinflammatory cytokine, IL-1. A recombinant human IL-1 receptor antagonist, (anakinra, Kineret™), first approved by the FDA in 2001 for the treatment of RA, provides a proven treatment for these diseases. The effectiveness of blocking IL-1 in NOMID was demonstrated by the findings that treatment with anakinra in patients with NOMID resulted in immediate resolution of the skin rash, and the symptoms returned when the IL-1 inhibitor was withdrawn. In patients with NO-MID, blocking IL-1 helped restore hearing and vision in some patients but had no effect in others. However, IL-1 inhibitors did not help prevent the growth of bony lesions in the knees. Early diagnosis and active treatment with an IL-1 inhibitor, such as anakinra, can reduce and perhaps prevent the development of organ-specific damage and disability.²⁵

IL-1 β plays a central role in several inflammatory diseases, including RA, CAPS, and gout, said Neil Stahl of Regeneron Pharmaceuticals, Inc. Rilonacept, a drug that is approved for treating CAPS and is now in Phase-3 trials for gout, is a receptor-Fc fusion protein that traps and promotes clearance of IL-1.²⁶ It is very specific for IL-1 and has a very high affinity for this cytokine. Rilonacept and IL-1 β form a complex that prevents the biological activity of this cytokine. Stahl and his colleagues measured the levels of circulating IL-1 in several diseases and found that IL-1 β levels are highest in patients with CAPS (FCAS), followed by gout and then RA. Normal healthy volunteers appear to have very low IL-1 β synthesis.

The Regeneron group concluded that studying IL-1 β :Rilonacept complex levels may prove useful in identifying IL-1 β “driven” diseases and therefore diseases that are more responsive to IL-1 inhibition. Other scientists at Regeneron, led by Allen Radin, are exploring the use of Rilonacept as a treatment for chronic gouty arthritis, a rare subset of the gout spectrum that is resistant to the standard drugs that are used to treat gout.²⁶

Use of Bioengineering to Improve the Bioactivity and Tissue-Specific Targeting of Cytokines as Drugs

Although many cytokines have defined biological activities that can be harnessed to treat certain diseases, there are at least two major challenges regarding the clinical use of cytokines as therapeutic agents. First, most cytokines have short half-lives (typically a few hours at most) when injected *in vivo*, and second, they often induce many undesired side effects, because receptors for most cytokines are broadly expressed on many different cells/tissues throughout the body. In their native form, cytokines are usually eliminated rapidly, both by receptor-mediated uptake as well as by enzymatic inactivation by proteases. The short half-lives of these biologic agents significantly limit their efficacy. Increasing the stability of these proteins would allow these agents to be given less frequently and at lower doses. Consequently, scientists have developed several methods to extend the half-life of cytokines *in vivo* and target them more selectively to specific tissue/organs.

Polyethylene Glycol Extends the Half-Life of Cytokines

The addition of polyethylene glycol (PEG) to proteins can greatly increase their half-life in the body and enhance other pharmacologically important properties such as their solubility. “Pegylation” was originally pioneered by Abraham Abuchowski of Prolong Pharmaceuticals, Inc., while he was a Ph.D. student at Rutgers University.²⁷ It is now a

standard and highly accepted method in the biopharmaceutical industry that is widely used to prolong the half-life of many protein therapeutics.

Pegylation has a number of benefits. It is nontoxic and increases the circulating half-life of many drugs, thereby reducing the number of doses and the frequency of dosing. Adding PEG causes molecules to become more water soluble because PEG readily binds water, creating a hydrodynamic shell that though it diminishes binding of a cytokine to its receptor does not fully block binding and with the increased longevity of the product produces a more sustained effect. Numerous chemical methods have now been developed to facilitate attachment of PEG moieties to both protein and nonproteinaceous molecules.

Cytokine Delivery Using Food-Grade Bacteria

An innovative and novel delivery system that is now being evaluated clinically involves oral delivery of therapeutic peptides or proteins via genetically engineered bacteria. Pioneered by Dr. Lothar Steidler and his team at ActoGeniX NV in Belgium, these noninvasive, noncolonizing, food-grade bacteria can secrete bioactive proteins or peptides into the gastrointestinal tract. These ActoBiotics are produced by a type of bacteria called *Lactococcus lactis* that are engineered to express a specific cytokine(s).²⁸

The company is using this novel delivery method to deliver recombinant human IL-10 to the gut as a possible new treatment of IBD.²⁹ This cytokine was tested in the past for treatment of IBD, but it was administered as a parenteral bolus either intravenously or subcutaneously. In view of its short half-life, the widespread expression of IL-10 receptors, and the systemic route of administration, the protein probably did not reach the critical target tissue, the mucosal lining of the gut. In the clinical trials of recombinant human IL-10 that were performed in the mid-1990s, parenteral administration of IL-10 at high concentrations proved to be ineffective. However, the use of *Lactococcus* to deliver IL-10 orally may now provide an improved method to deliver this cytokine more selectively to the gut where it may act to suppress inflammation.

Final Comments

As discussed by Dr. Steven Kozlowski of the FDA Office of Biotechnology Products, a number of technical hurdles still limit the full clinical potential of cytokine-related therapies.³⁰ However, much has been learned during the last 20 years or so as a result of the initial attempts to use recombinant cytokines as therapeutic agents for various clinical indications.

Clearly, much more research needs to be done before we fully understand how cytokines can be most effectively used as therapeutic agents for treating the spectrum of human diseases. However, cytokines and cytokine inhibitors continue to provide a rich pipeline of novel therapeutic agents for treating various cancers, autoimmune diseases, and infectious diseases. On the basis of discussions that took place during this conference, there was general agreement that a greater understanding of the downstream, intracellular, signaling cascades and cytokine-inducible genes will facilitate development of more judicious clinical use of cytokines as therapeutic agents. Several of the speakers at this conference pointed out that many new biomarkers have now been discovered that may provide very useful tools to

identify specific patient subpopulations that would be predicted to have therapeutic benefit from a particular cytokine or cytokine antagonist and to monitor therapeutic efficacy.

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Figure 1.

Interferons are a group of cytokines that can induce potent antiviral activity in many cell types. The type I, type II, and type III interferons bind to distinct receptor complexes on the cell membrane. Signal transduction induced by the binding of interferons to their cognate receptors induces expression of interferon-stimulated genes (ISGs). The proteins encoded by these genes in turn mediate the antiviral activity of the interferons.