Cytokines: Lessons From the Past to a Brighter Future in Therapeutics

Jean-Michel Dayer, MD, University Hospital, Faculty of Medicine, Geneva, presented the Fred Wyss lecture at the 2007 EULAR congress in Barcelona. He gave a brief history of the advancement of cytokine based therapy and discussed the role of these agents in the future.

The development of what Prof. Dayer referred to as the "cytokine community" has followed a repeatable sequence. At first, an individual cytokine is identified and associated with a particular function. Later, it is found to have many other functions. At some point, the cytokine is cloned with the assumption that there will be one function associated with one factor, but the result is often that new, unexpected members of the cytokine family are identified and novel functions are revealed. This, in turn, leads to both clinical and biological surprises, species problems, side effects, and antagonists and agonists within the same family. The TNF, IL-1, IL-6 families are excellent examples of this process.

TNF was discovered many years ago as a serum factor that causes necrosis of tumors in mice. Years later, the TNF receptor was shown to be expressed by mammalian cells. This led to the discovery of a superfamily of transmembrane proteins and the identification of gene families that include 18 ligands and 28 receptors.

TNF has been found to induce collagenase and PGE_2 in human synovial cells from patients with RA [Dayer J-M et al. *J Exp Med* 1985] and to induce bone resorption [Saklatvala J et al. *Nature* 1986]. It was determined to be involved in collagen-induced arthritis [William RO et al. *Proc Natl Acad Sci* 1982] and was detected in the biological fluids of rheumatoid arthritis (RA) patients [Saxne T et al. *Arthritis and Rheum* 1988]. A key observation was that TNF is very important in the hierarchy of the cytokines, since antibodies to TNF decrease other downstream cytokines [Brennan FM et al. *Lancet*, 1989]. Using a chimeric monoclonal antibody to TNF-α as a treatment for RA leads to impressive clinical results [Elliott JM et al. *Arthritis Rheum* 1993].

The developmental history of cytokines and IL-1 followed a path similar to the TNF developments. Many functions were attributed to IL-1, such aslymphocyte activating factor (LAF) osteaoclast-activating factors (OAF), endogenous pyrogen (EP) and related to RA mononuclear cell factor (MCF) inducing collagenase and PGE_2 [Dayer et al. *Science* 1977]. IL-1 biology still leads to some surprises. For example, in a mouse model, local hippocampal over expression of IL-1 beta in an Alzheimer's diseased transgenic mouse resulted not in the expected exacerbation of the amyloid beta plaque deposition common in Alzheimer's disease, but instead in plaque amelioration [Solomon S et al. *J Clin Invest* 2007]. IL-1 receptor antagonists (IL-1Ra) was shown to inhibit insulin production in cultured rat pancreatic islets [Dayer-Métroz MD et al. *J of Autoimmunity* 1989]. Of great interest was the finding that the expression of the IL-1Ra was reduced in the pancreatic islets of patients with type 2 diabetes mellitus. The blockade of IL-1 with anakinra improved glycemia and beta-cell secretory function and reduced markers of systemic inflammation [Larsen CM et al. *N Eng J Med* 2007].

Prof. Dayer started his EULAR lecture by posing the question "Why does a patient's fever regress spontaneously?" He then proposed the following hypothesis: An insult to the system causes an immuno-inflammatory reaction; cytokines are released that cause pain, tissue destruction, and inflammation. A defensive anti-immuno-inflammatory reaction occurs, including the release of anti-cytokines that block the inflammation

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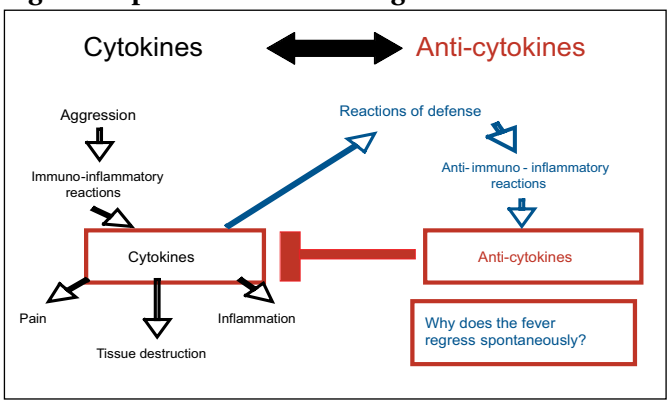
Annual European Congress of Rheumatology EULAR 2007

caused by the originally released cytokines (Figure 1), thus causing the fever to regress.

Figure 1: Spontaneous Fever Regression.



Proof of this can be found in a study conducted by Prieur et al, in which IL-1 activity and inhibition were studied in serum and urine from 9 patients with systemic juvenile chronic arthritis (S-JCA) [Prieur A et al. *Lancet* 1987]. In afebrile patients, IL-1 bio-activity was normal or high. Serum from 2 afebrile S-JCA patients taken during a period of severe disease activity had an enhancing effect on the bio-activity of exogenous IL-1. In contrast, febrile patients' serum and urine IL-1 bio-activity was low, apparently reflecting the presence of a strong inhibitor of IL-1 activity measured by the inhibition of prostaglandin E2 production by synovial cells. This inhibition was greatest at the time of peak temperature, suggesting the possibility of feedback regulation during fever. Recently, anakinra has been shown to be successful in treating patients with adult-onset Still's disease [Fitzgerald JD et al. *Arthritis Rheum* 2005; Vasques G et al. *Ann Rheum Dis* 2005] and, surprisingly, in acute gout [So A et al. *Arthritis Res Ther* 2007].

Where are the initial events in RA? Locating the site of disease-initiating events is still under debate. Is it at the systemic level (extra-articular site), or in the bone marrow, or locally (articular site)? Support for systemic localization comes from Binstadt and colleagues [*Nat Immunol* 2006]. Using observations in the K/BxN murine arthritis model, they uncovered novel pathways underlying the site-specific localization of inflammation driven by immune complexes and triggered by sensitization to non-specific Ag at an extra-articular level. Such a hypothesis has been reviewed by Pitzalis C et al. [*Trend in Immunology* 2006].

Within both the bone marrow and the synovium, fibroblastic stromal cells play an important role in supporting the differentiation and survival of normal cells. They also contribute to the pathologic processes. A possible argument for the localization of the initiating event in the bone marrow could be the presence of nurse cells within the synovium that foster inflammation. These nurse cells may contribute to the localization of inflammation within specific joints. It has also been noted that fibroblastic stromal cells from epiphyseal bone marrow can migrate into the joint space, forming synovial tissue in collagen-induced arthritis [Ochi T et al. *Arthritis Res Ther* 2007].

What about the local articular site? The overgrowth of synovial tissues is critical in the pathogenesis of rheumatoid arthritis (RA). The expression of Synoviolin (SYN), an E3 ubiquitin ligase, is upregulated in arthritic synovial fibroblasts and is involved in the overgrowth of synovial cells during RA. The proinflammatory cytokines IL-1β and TNFα induce the overgrowth of synovial cells by upregulating SYN expression via the Erk1/-ETS1 pathway [Gao B et al. *Arthritis Res Ther* 2006]. Another molecule, Cadherin-11, strongly determines the behavior of synovial cells in their proinflammatory and destructive tissue response in inflammatory arthritis [Lee DM. *Science* 2007].

Prof. Dayer also spoke briefly about the new research affecting cytokine understanding, such as cell to cell contact between T lymphocytes and monocytes for IL-1/TNF production and the environmental influence of Apolipoprotein A_1-HDL blocking [Hyka N. *Blood* 2001; Dayer JM et al. *Autoimmune Res* 2004]; on the production of cytokines, and the role of adipose tissue.

Adipocytokines are cytokines secreted by adipose tissue, the source of production and site of action of several pro- and anti-inflammatory cytokines. White adipose tissues are the major producer of the anti-inflammatory IL-1Ra [Juqe-Aubry CE et al. *J Clin End Metabol* 2004]. Some adipocytokines such as adiponectin and leptin affect immune and inflammatory functions. A new proinflammatory adipocytokine (Visfatin) has recently been identified as an adipocytokine that activates human leukocytes and induces cytokine production [Moschen AR et al. *J Immunol* 2007].

Emerging cytokine targets in RA, eg, IL-6, IL-15, and IL-32, are in multiple stages of development. New cell therapies using T-regulatory cells, hematopoietic stem cell transplantation, and mesenchymal stem cells are also in development.

New treatments based upon vaccination with cytokines are emerging. An anti-cytokine induction of autoimmune protection against both acute and chronic hTNFα exposure has been demonstrated. Thus, an

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Humans lack uricase, the urate oxidase enzyme that converts uric acid to allantoin. When given intravenously uricase can reduce serum uric acid levels to nearly zero. There are reports of dramatic reduction in tophi using Rasburicase (a pegylated form of Aspergillus-derived uricase licensed for tumour lysis syndrome), but immunogenicity limits its repeated use. Therefore, there are current efforts to develop a less immunogenic form of mammalian uricase for repeated use in gout [Ganson NJ et al. *Arthritis Res Ther* 2006].

There are recent data from the UK showing that a minority of gout patients receive education, including dietary and other lifestyle advice and that only approximately one third are given ULT [Mikuls TR et al. *Ann Rheum Dis* 2005; Roddy et al. *Ann Rheum Dis* 2007. In press]. Of these, almost all are on allopurinol at a standard dose of 300mg/day, which is an insufficient dose for many patients, meaning that the majority of gout patients do not experience "cure" [Roddy et al]. Therefore education on the principles of long-term gout management and optimization of currently available treatments alone could have a major impact on improving the outcome of this common, painful, inflammatory arthritis.

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effective and safe vaccination against a human cytokine may be achievable [Le Buanex H et al. *Proc Natl Acad Sci USA* 2006].

A number of peptide, and peptidomimetic-based approaches (such as TCR-peptide vaccines and peptides derived from heat shock protein), and antisense oligonucleotide are currently being tested in animal models to treat inflammatory arthritis.

"We have learned, and will continue to learn, a great deal about cytokines, a remarkable class of potential disease altering agents that will play a major role in future therapeutics, and lead to more 'ad personam' treatment depending upon the subtypes of diseases and the gene status." Prof. Dayer concluded.

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Clinical Presentation

Clinical hallmarks include Heberden's and Bouchard's nodes, and/or bony enlargement with or without deformity affecting characteristic target joints (DIPJs, PIPJs, thumb-base, and index and middle MCPJs).

Associated Risks/Subsets

Patients with polyarticular OA of the hand are at increased risk of OA of the knee, hip, and other common target sites and should be assessed and examined accordingly. Recognised subsets with different risk factors, associations and outcomes (requiring different assessment and management) include IPJ (with or without nodes), thumb-base, and erosive OA.

Diagnosis

The differential diagnosis for OA of the hand is wide. The most common conditions to consider are psoriatic arthritis; rheumatoid arthritis, gout, and hemochromatosis. Radiographs provide the gold standard for morphological assessment of OA of the hand. Blood tests are not required for diagnosis but may be required to exclude co-existent disease.

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Dr. Gossec commented, "Measuring joint space width, in particular in the semi-flexed knee, has been shown to be the most reliable and responsive way to determine structural damage severity in knee OA trials, since overall, reliability and responsiveness were higher for JSW (in particular on semi-flexed view) than for the other scoring techniques."

Prof. Wim B. van den Berg, MD, University Hospital Nijmegen, The Netherlands, provided a glimpse into the future of OA treatment via animal model studies that are exploring novel therapeutic targets in OA. Among these are IL-1 and the role of activated macrophages and degradative enzymes such as ADAMS5 and stromelysin. Novel receptors currently being investigated include the toll like receptors (TLRs) and the receptors of advanced glycation end products. TLRs are expressed on chondrocytes and synoviocytes. When activated, they drive the degradative enzymes. Advanced glycation end products are the result of non-enzymatic glycation of proteins, such as collagen. They accumulate with age and result in pathologic stiffening of cartilage and extracellular matrix.

These ongoing investigative efforts hold promise for the development of novel drugs both for the management of pain as well as retarding the OA process.