Cytokine Network and Its Manipulation in Rheumatoid Arthritis

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Abstract
Studies of the inflammatory process in the inflamed synovium from rheumatoid arthritis patients have shown an intricate network of molecules involved in its initiation, perpetuation and regulation trial balances the pro- and anti-inflammatory process. This system is self-regulating though the action of anti-inflammatory and pro-inflammatory cytokines cytokine receptor antagonists and naturally occurring antibodies cytokines. Inflammatory synovitis in rheumatoid arthritis (and possibly in other inflammatory arthritides) appears to be the result of an imbalance in the cytokine network with either an excess production of pro-inflammatory cytokines or from inadequacy of the natural anti-inflammatory mechanisms. Using this knowledge the newer therapeutic approaches to RA and other inflammatory arthritides are being aimed at correcting this imbalance. Monoclonal antibodies to INF-α (humanised form of this is called infliximab), soluble TNF-α receptors (etanercept) are already in clinical use and adalimumab (humanised TNF-α antibody). IL-1Ra is undergoing clinical trials. Other promising therapeutic agents that could regulate the cytokine network are in various stages of laboratory and clinical evaluation. These studies promise to yield therapeutic targets that could dramatically change the way inflammatory diseases would be treated in the future. The now established efficacy of infliximab and etanercept in inflammatory arthritides could be considered just a glimpse of the exciting scenario of the future.

INTRODUCTION
Joint damage in rheumatoid arthritis (RA) is mediated through immunological mechanism. However, for decades, the exact steps involved in the initiation and perpetuation of synovial inflammation and bone erosion in RA remained a mystery. Using RA as the prototype inflammatory arthritis this article reviews some of the complex processes that lead to joint inflammation. The subject has been extensively reviewed in the recent past. This article has been written based upon information gained from these reviews, Articles similar to the present one have been written in the recent past.

Cytokines, the small peptides with potent short-range biological actions, appear to play a key role in the process of joint inflammation; it would appear that this complex process includes several interdependent interactions between pro-inflammatory and anti-inflammatory mechanisms. Recent years have seen therapeutic exploitation of this understanding of the development of an array of etiological agents that can manipulate and modulate the aserant immune processes. These advances became possible only because of the development of the technique for the in vitro culture of dissociated unpurified synovial cells from actively inflamed RA joints. Prof. R. N. Maini’s laboratory in London was able to establish the culture of such cell mixtures (synovial cells, lymphocytes, macrophages, fibroblasts etc.) that continuously released cytokines in vitro without extrinsic stimulation. This biological model made it possible to study the exact steps involved in synovial inflammation. In addition, certain biological tools that include the hybridoma technology for the production of monoclonal antibodies and the recombinant DNA technology for the production of ‘designer’ molecules have been crucial in this field of research.

Cytokine biology
Cytokines are small peptides that have been shown to be important mediators of several fundamental biological processes that include (i) inflammation (ii) tissue repair (iii) cell growth (iv) fibrosis (v) angiogenesis and (vi) immune response. There are many families of cytokines. Over hundred molecules have already been identified and many more are in the process of being identified. They are characterised by the following: (i) Upon activation they can be produced by almost any cell in the body; (ii) Majority of them act locally (exception interleukin-6 that acts systemically); (iii) They act on high affinity receptors present on other cells. Therefore, their biological actions are extremely potent (iv) Cytokine
inhibitors are naturally present concomitantly and serve as regulators of the response.

**Regulatory effect of cytokines in inflammation**

With the available technologies in the '80s, it became possible to study the inflammatory joint fluid in RA. One of the earliest reports established that RA synovial cultures released more IL-1 than osteoarthritis synovial cultures. However, the issue appeared complicated because many other cytokines were also up-regulated in actively inflamed joints. Therefore, it was considered essential to understand the regulatory mechanisms involved in the production of these cytokines. This was achieved by using monoclonal antibodies against a battery of key cytokines that were considered crucial in triggering the inflammatory cascade. Painstaking research yielded results and Prof. Maini’s group found one such monoclonal antibody with specificity against tumor necrosis factor-α (TNF-α) that was able to dramatically suppress the production of IL-1, the most abundant cytokine in inflamed RA joint.\(^\text{17}\) This observation was the first step towards understanding the coordinately regulated production of cytokines involved in causing inflammation in RA joint. Further studies established that suppression of TNF-α not only down-regulated IL-1 production but also several other cytokines found in abundance in inflamed RA joints (e.g. GM-CSF, IL-6, IL-9).\(^\text{20,21}\)

1. **Cytokine balance in inflammation:**

   In the synovium of the inflamed joints there is a cytokine balance. It is based upon feedback mechanisms as well as opposing actions of different cytokines. With regard to inflammation, there are clearly two groups of cytokines namely (i) the pro-inflammatory and (ii) the anti-inflammatory cytokines (Table 1).

   The anti-inflammatory cytokines block the action of pro-inflammatory cytokines, proteolytic enzymes (that are involved in the cartilage damage), and up-regulate the IL-1 receptor antagonist (IL-1Ra), a major anti-inflammatory cytokine. The pro-inflammatory cytokines have exactly the opposite action.

   Interestingly the CD4 +ve T helper (Th) lymphocytes have two main phenotypes TH1 and Th2 subsets that are characterised by two distinct cytokine profiles.

   A third subset called Th0 appears to be the progenitor of these two subsets. Its cytokine profile is intermediate. The Th phenotype of the lymphocyte determines the function of the T cell subsets. Thus, the Th1 cells predominantly mediate the cell-mediated immune response (CMI) while the Th2 cells mediated the humoral (antibody) immune response. On the basis of these observations the predominant immune response in RA appears to be that of Th1 class while in SLE, a disease characterised with exaggerated production of autoantibodies, it is mainly characterised by exaggerated Th2 response.

   The anti-inflammatory cytokines suppress the Th1-type response characteristic of RA. These observations predict a possible therapeutic role of the Th2-type anti-inflammatory cytokines in the control of RA. They also predict that these cytokines may not be effective, or may even be harmful, in SLE. Table 2 gives the predominant cytokine-type of the Th lymphocytes and their action.

   Among the various cytokines IL-1, IL-8 and TNF-α have been characterised as the major proinflammatory cytokines. These could, therefore, be major targets for therapeutic manipulation. As mentioned above, the Th2-type cytokines namely IL-4, IL-10, IL-13 inhibit the Th1-type response thus suppressing the production of pro-inflammatory cytokines, block inflammatory cell emigration, stimulate the production of other anti-inflammatory molecules (e.g. IL-1 Ra) and inhibit the release of proteolytic enzymes that are mainly responsible for cartilage and bone destruction in RA. There are several additional molecules in the chemokine-monokine and growth factor family that have also been shown to be anti-inflammatory. These include IFN-γ-inducible protein 10 (IP-10) and platelet factor 4 (PF4). Therefore, these could also be possible therapeutic targets in RA.

2. **Major cytokines, chemokines and growth factors in synovial inflammation:**

   As mentioned in the beginning large bulk of the information has been derived from the study of rheumatoid synovium. These studies have shown that IL-1 and TNF-α are the most important proinflammatory cytokines in rheumatoid synovium. They have overlapping actions including local inflammation, enhancing adhesive properties of the inflammatory cells, causing angiogenesis, and bone resorption. It seems that while TNF-α is more concerned with inflammation, IL-1 is the main cytokine causing cartilage destruction and bone resorption that leads to osteoporosis. Both of these cytokines are mainly produced by monocyte-macrophage lineage of cells. IL-1 is also produced by B cells, endothelial cells and activated T cells.

   IL-6 is another important proinflammatory cytokine with several effects overlapping with that of IL-1 and TNF-α. Gold therapy has been shown to lower the IL-6 levels. Anti-IL-6 therapy, therefore, could be one of the possible therapeutic targets.

   IL-11 has disparate pro-inflammatory and anti-inflammatory effects. Thus, is seems to block production of TNF-α and matrix metalloproteinasises (MMP) but, is involved in the causation of osteopenia.

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**Table 1:** Pro-inflammatory and anti-inflammatory cytokines in RA joint:

<table>
<thead>
<tr>
<th>Pro-inflammatory cytokines</th>
<th>Anti-inflammatory cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, IL-1, IL-8, IFN-α, IL-2</td>
<td>IL-4, IL-10, IL-13, IL-1 receptor antagonist, soluble TNF-receptor</td>
</tr>
</tbody>
</table>

**Table 2:** The phenotypes of T-helper cells (Th), their cytokine profiles and their biological action.

<table>
<thead>
<tr>
<th>Th phenotypes</th>
<th>Cytokine profiles</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 lymphocytes</td>
<td>IFN-α, IL-2, IL-12</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>Th2 lymphocytes</td>
<td>IL-4, IL-5, IL-10, IL-13</td>
<td>Anti-inflammatory</td>
</tr>
</tbody>
</table>
II-4, IL-10 and IL-13 neutralize the action of pro-inflammatory cytokines mainly by increasing the production of IL-1Ra (soluble receptor antagonist). Therefore, these cytokines could be used as therapeutic agents.

There are several other cytokines that include IL-12, IL-15, IL-16, IL-17 and IFN-γ that are closely involved in inflammation and its regulation. Some of them could be important therapeutic targets. Similarly there are several other adhesion molecules, chemokines and growth factors with regulatory role. Some of them may turn out to be important therapeutic targets.

Cytokines however, have both pro- as well as anti-inflammatory effect in different situations. Many of the effects of IL-6 overlap with that of IL-1 and TNF-α.

3. **Natural cytokine modulators:**

   Studies of the molecules involved in inflammation have established that there are a number of naturally occurring molecules that act as regulators of this process. Thus there are naturally occurring anti-cytokine antibodies against IL-1, IL-6, TNF-α and IFN-γ. One such artificially produced anti-TNF-α (infliximab), is already in clinical use. Similarily, soluble cytokine receptors are also found in the body, especially at the site of inflammation that seems to block the action of the cytokine. These may be important therapeutically. One such molecule namely etanercept (soluble receptor of TNF-α), is already in clinical use. Naturally occurring cytokine receptor antagonists have also been found. One of them is IL-1 Ra that has been shown to antagonize most of the proinflammatory effects of IL-1. It would appear to be an excellent therapeutic agent.

4. **The possible therapeutic targets:**

   The above discussion on cytokine biology clearly brings up the exciting theoretical possibility of modulating this network for therapeutic purposes in inflammatory diseases. Although there seem to be innumerable such possible targets, studies indicate that the following sites of attack are likely to be therapeutically important:

   - TNF - blockage
   - Interleukin-1 blockage
   - Interleukin-6 blockage

   **Cell surface-targeted therapies:**
   - Anti-adhesion molecules (ICAM-1)
   - Anti-CD4, -CD5, -CD7, -CD25, -CD28, -CD-52
   - Interleukin-2-fusion protein

   Figure 1 diagrammatically depicts the pro-inflammatory and regulatory cytokines in the inflamed synovium in RA along with some possible therapeutic targets.

**Conclusion**

In this article, the mechanism of induction and perpetuation of the inflammatory process in inflamed synovium from rheumatoid arthritis patients has been reviewed from the standpoint of the involvement of the various cytokines. The available information has shown that there is an intricate network of molecules involved in its initiation, perpetuation and regulation that balance the pro- and anti-inflammatory process. The self-regulatory nature of this network has been highlighted with a balance between the action of anti-inflammatory and pro-inflammatory cytokines, cytokine receptor antagonists, and naturally occurring antibodies to cytokines. It would appear that the inflammatory synovitis in rheumatoid arthritis (and possibly in other inflammatory arthritides) could be the result of an imbalance in the cytokine network with either an excess production of pro-inflammatory cytokines or from inadequacy of the natural anti-inflammatory mechanisms. This knowledge could be exploited for the manipulation of the cytokine network with the aim of correcting this imbalance. Three most promising products appear to be the use of monoclonal antibodies to TNF-α (infliximab), soluble TNF-α receptors (etanercept), and IL-1Ra (undergoing clinical trials). Other promising therapeutic agents that could regulate the cytokine network are in various stages of laboratory and clinical evaluation. These studies promise to yield therapeutic targets that could dramatically change the way inflammatory diseases would be treated in the future. Efficacy of infliximab and etanercept in inflammatory arthritides appears to be just the beginning in the exciting field of biological therapies for complex diseases of human beings.
REFERENCES


