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Review

## PRO- versus ANTI-INFLAMMATORY CYTOKINES: MYTH OR REALITY

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**Abstract** - Inflammation is characterized by an interplay between pro- and anti-inflammatory cytokines. Cytokines are commonly classified in one or the other category: interleukin-1 (IL-1), tumor necrosis factor (TNF), gamma-interferon (IFN- $\gamma$ ), IL-12, IL-18 and granulocyte-macrophage colony stimulating factor are well characterized as pro-inflammatory cytokines whereas IL-4, IL-10, IL-13, IFN- $\alpha$  and transforming growth factor- $\beta$  are recognized as anti-inflammatory cytokines. In this review, we point out that this classification is far too simplistic and we provide numerous examples illustrating that a given cytokine may behave as a pro- as well as an anti-inflammatory cytokine. Indeed, the cytokine amount, the nature of the target cell, the nature of the activating signal, the nature of produced cytokines, the timing, the sequence of cytokine action and even the experimental model are parameters which greatly influence cytokine properties.

**Key words:** Inflammation, interleukin, chemokine, macrophages, neutrophils, endothelial cells

### INTRODUCTION

Cytokines play an important role during the inflammatory process. Two cytokines, namely interleukin-1 (IL-1) and tumor necrosis factor (TNF) orchestrate the inflammatory response and initiate a cascade of mediators which are directly responsible for the various events associated with inflammation (e.g. increased vascular permeability, chemoattraction of circulating leukocytes, proteolysis...). Other cytokines such as IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF) amplify the release of IL-1 and TNF, thus favoring the inflammatory process. This is also the case for gamma-interferon (IFN- $\gamma$ ) the production of which is induced by IL-12 and IL-18. While the cytokines mentioned above are classified as "pro-inflammatory cytokines", IL-4, IL-10, IL-13, interferon-alpha (IFN- $\alpha$ ) and transforming growth factor- $\beta$  (TGF- $\beta$ ) are recognized as anti-inflammatory cytokines because of their ability to inhibit the release of pro-inflammatory cytokines, to induce the production of IL-1 receptor antagonist (IL-1ra) and the release of soluble TNF receptor (sTNFR) and to limit some of the pro-inflammatory activities of IL-1 and TNF. However, the events occurring during inflammation are not as simplistic

as an interplay between pro- and anti-inflammatory actors. Indeed, they are far more complex ! In this short review we will provide some examples which illustrate the fact that each of these cytokines offers a "half angel - half devil" aspect and none can be simply labelled either "pro" or "anti".

### A TOO SIMPLISTIC DICHOTOMY

René Magritte, the surrealist Belgian artist, painted a pipe on a picture and wrote "Ceci n'est pas une pipe" (*This is not a pipe*). It is becoming more and more frequent to find reports reminiscent of this concept: e.g. "TNF is not a pro-inflammatory cytokine". For example, in their report entitled "TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination" Liu *et al.* (42) showed that in response to injection of myelin oligodendrocyte glycoprotein, TNF-deficient mice of different genetic backgrounds displayed a multiple sclerosis-like disease with a higher incidence, a higher mortality, a longer duration and a more severe autoimmune disease than their wild type counterparts. Similarly, in an experimental model of collagen-induced arthritis, it was found that blocking the activity of IFN- $\gamma$  (either by anti-IFN- $\gamma$  antiserum or by using IFN- $\gamma$  receptor

knock-out mice) resulted in an accelerated onset of the disease (70). These results suggested that IFN- $\gamma$ , instead of being a pro-inflammatory cytokine, was rather involved in counteracting the development of the disease in this experimental model. As well, one can assert that "IL-10 is not an anti-inflammatory cytokine". Evidence comes from *in vivo* works in which pro-inflammatory or immunostimulating activities have been reported for IL-10. This is the case for autoimmune diabetes whose onset and development are accelerated in transgenic mice overexpressing IL-10 in pancreatic islets (52,74). Also, IL-10 treatment accelerates allograft rejection of islet cells (77) and heart (56). In a model of endotoxin-induced uveitis, intra-peritoneal injection of IL-10 potentiated the ocular inflammation (59). Finally, in a tumor model, IL-10 was reported to favor tumor rejection (6) and using transfected mouse mammary adenocarcinoma cells expressing IL-10, Di Carlo *et al.* (20) showed that the tumor growth area was associated with an enhanced level of the chemokine "monocyte-chemoattractant protein-1" (MCP-1) and of inducible nitric oxide synthase (iNOS), an enhanced expression of VCAM-1 and ELAM-1 adhesion molecules and an enhanced recruitment of leukocytes as compared to mice receiving the parent adenocarcinoma. This parallels the fact that IL-10 induces E-selectin expression on small and large blood-vessel endothelial cells (71).

We will now review few parameters which influence the behavior of the different cytokines and may explain why, depending upon the situation, both pro- and anti-inflammatory properties can be described for the same mediators.

### THE AMOUNT OF CYTOKINE

The intensity of the inflammatory response is associated with different physiological events which correlate with the levels of the produced cytokine. The pro-inflammatory cytokines are the most necessary mediators to set-up an anti-infectious response; however, an exacerbated production of these cytokines may be deleterious and even lead to death when used in animal models and be associated with poor outcome in human pathologies such as sepsis. On the other hand, while anti-inflammatory cytokines are a prerequisite to control the cascade of pro-inflammatory mediators, their excessive production is associated with a severe immune depression as observed in patients following trauma or major surgery. Consequently, an increased sensitivity to nosocomial infections is observed in these patients.

The amount of a given cytokine clearly influences its

properties. The best example is given with TGF- $\beta$  (9): in addition to its role in controlling inflammation, TGF- $\beta$  restrains cell proliferation and controls turnover of the extracellular matrix. At high concentration, TGF- $\beta$  suppresses cell proliferation and stimulates the production of pathological amounts of extracellular matrix (fibrosis) whereas at low levels, TGF- $\beta$  predisposes to excessive cell proliferation, atherogenesis or reduced production of extracellular matrix and impaired wound healing. Similarly, it has been reported that some effects of TNF were influenced by the amount of this cytokine used in the experimental model. Low doses were found to induced angiogenesis whereas high concentrations were associated with an inhibition of angiogenesis (23). Moreover, in an elegant experimental model of arthritis induced by the injection of acidified type II collagen, it was demonstrated that low amounts of IL-12 were pro-inflammatory whereas 100 fold higher amounts were associated with an anti-inflammatory process (37). Injection of 5 ng of IL-12 a day increased the severity of the disease, a property which was essentially TNF-dependent whereas treatment with 500 ng a day significantly decreased the mean arthritis index of the pathology, a phenomenon which was essentially IL-10-dependent. Interestingly, only large amounts of IL-12 induced circulating corticosterone.

### THE NATURE OF THE TARGET CELL

The anti-inflammatory properties of our quintet of anti-inflammatory cytokines have essentially been coined with monocytes/macrophages used as target cells. There are numerous examples which illustrate that the story might be completely different with other target cells. Thus, IL-10 was first identified and defined as a cytokine capable to repress the production of IFN- $\gamma$  by Th1 clones (25), but more recently it was demonstrated that IL-10 enhanced the production of IFN- $\gamma$  by NK cells (63), increased the intracellular expression of IFN- $\gamma$  and IL-2 in CD8<sup>+</sup> T-cells in combination with IL-2 after antigen stimulation (60) and increased the number of IL-2 secreting CD4<sup>+</sup> T-cell clones (40). Furthermore, IL-4 and IL-10 which inhibit the LPS-induced production of IL-8 by macrophages, amplify that of endothelial cells (18). The different efficiency to inhibit IL-8 production depending on the nature of the target cells has also been reported for INF- $\alpha$  which limits this production by LPS-activated peripheral blood mononuclear cells and by TNF- $\alpha$ -stimulated bone marrow stroma cells but which is inefficient when acting on LPS-activated neutrophils (2). While IL-13 diminishes chemokine production by

activated macrophages, it induces the synthesis of MCP-1 by endothelial cells (29). While TGF- $\beta$ 1 limits the production of IL-1 $\alpha$  and IL-8 in macrophages, it induces them in epithelial cells (38). While IL-10 can repress the production of nitric oxide (NO) by macrophages or keratinocytes (4,13), it does not modify NO release by mesangial cells (26) and even enhances the production of NO by bone marrow derived macrophages and osteoclasts (7,65). Acting on bone marrow derived mast cells, IL-10 synergized with c-kit ligand and LPS to increase the production of cyclooxygenase type 2 and PGD<sub>2</sub> as well as the expression of IL-6 mRNA (51). When addressing the regulation of IL-1 $\beta$ -induced IL-6 production by astrocytes, Poussat *et al.* (55) showed that IL-10 but neither IL-4 nor dexamethasone possessed inhibitory properties.

The target cell status may modify its reactivity as well. Accordingly, IL-10 alone or in synergy with TNF enhances HIV replication and TNF production by HIV-infected T-cells or promonocytic cells (24,57). Most importantly, environmental parameters may also influence the reactivity of a given cell type. The best example is provided by the study of Pang *et al.* (53) who reported in chronic bronchial sepsis that IL-10 was able to inhibit the LPS-induced IL-8 production by circulating neutrophils but was unable to do so when the same assay was performed with sputum-derived neutrophils. Similarly, analysis of spontaneous NO generation by macrophages from inflamed, but not normal glomeruli, was down-regulated by the addition of IL-4 or TGF- $\beta$  (22).

Discrepancies have also been reported in terms of the induction of adhesion molecules. For example, IL-4 inhibits the IL-1- or TNF-induced expression of ICAM-1 and ELAM-1 on the surface of endothelial cells, but it induces ICAM-1 expression in human epithelial cells (64) and favors the expression of VCAM-1 on endothelial cells, allowing the adherence of basophils and eosinophils (62). On the other hand, IL-10 inhibits ICAM-1 expression on human Langerhans cells but not on keratinocytes, dermal endothelial cells or fibroblasts (12).

### THE NATURE OF THE ACTIVATING SIGNAL

The inhibitory capacity of the so-called anti-inflammatory cytokines may also depend on the nature of the triggering agent which acts simultaneously on the target cell. For example, we have shown that IL-4 and IL-10 repress the LPS-induced IL-8 production by neutrophils while this is not the case when neutrophils were activated

by TNF- $\alpha$  (47). Surprisingly, the production of IL-1ra by activated neutrophils did not reflect what was described for the inhibition of IL-8: we reported that IL-10 was not acting in synergy with LPS but was active when used simultaneously with TNF- $\alpha$  to further enhance the production of IL-1ra (46). In contrast, IL-4 amplifies the production of IL-1ra by neutrophils, independently of the nature of the activating signals. The studies on the modulation of the production of various chemokines led to a rather complex pattern. Thus, it has been reported that IL-4 did not affect the production of RANTES by IFN- $\gamma$ -activated human monocytes whereas it was capable to increase this production when the cells were activated with TNF- $\alpha$  (44). In the presence of IL-2, the production of IFN- $\gamma$  by splenocytes from scid mice was unchanged when the cells were cultured with IL-12 and TNF- $\alpha$  whereas this production was greatly inhibited when the cells were activated with heat-killed *Listeria monocytogenes* (67). When the proliferation of CD8<sup>+</sup> T-cells was monitored in the presence of IL-10, the proliferative response could be either reduced (in the presence of allogenic monocytes), or unchanged (in the presence of anti-CD3 antibodies) or even enhanced (in the presence of IL-2) (30). Studies on the induction of tissue factor on the surface of monocytes or endothelial cells also revealed major differences based on the nature of the activating signal: IL-4 and IL-13 fully inhibited the induction of the expression of tissue factor on the surface of endothelial cells activated with LPS, whereas there was no inhibition when IL-1 $\beta$  was used as the triggering agent (32). A totally different pattern was obtained when tissue factor expression was analyzed on the surface of monocytes.

### THE NATURE OF THE PRODUCED CYTOKINE

The capacity of a given cytokine to inhibit the production of others may also vary depending on the nature of these other cytokines. For example, TNF was surprisingly shown to be a potent inhibitor of IL-12 secretion from human monocyte-derived macrophages activated with either LPS or *Staphylococcus aureus* whereas no similar inhibitory activity was reported when addressing the production of IL-1 $\alpha$ , IL-1 $\beta$  and IL-6 (45). Similarly, the so-called anti-inflammatory cytokines do not inhibit the production of all cytokines. Thus, IL-10 reduces the production of IL-12 by CD40L-activated dendritic cells whereas it does not modify the production of IL-8 and TNF- $\alpha$  (11). We reported that in whole blood

samples activated by heat-killed *Streptococcus pyogenes*, IL-13 inhibited the production of IL-8 but was unable to modify that of TNF- $\alpha$  (45). In addition, when the effects of IL-4 were studied on monocytes cultured for 7 days, it was demonstrated that the LPS-induced IL-1 $\beta$  production was reduced whereas the TNF- $\alpha$  production was unaffected (31). Studying IL-1 $\alpha$ -activated human bone marrow stroma cells, IL-4 was also shown to enhance the IL-8 production but to inhibit that of leukemia inhibitory factor (LIF) (19). The field of chemokines offers numerous examples of different regulations induced by the same cytokine. For example, IL-4 acting on macrophages inhibits the production of IL-8 and MIP-1 $\alpha$  but favors the release of MCP-1, RANTES, AMAC-1 and C10. A completely different profile might be found when considering another target cell. Thus, IL-4, when acting on endothelial cells favors the production of IL-8 and MCP-1 but limits that of RANTES. A similar heterogeneity in terms of responsiveness has also been reported with IFN- $\gamma$  which enhances the production of IP-10 and RANTES by macrophages but inhibits the production of GRO, MIP-1 $\alpha$ , MIP-1 $\beta$  and AMAC-1.

### THE TIMING

The fact that a mediator exerts an inhibiting or, on the contrary, an enhancing effect may also be linked to the timing of its exposure to the target cells. For example, IL-4 and IL-13 inhibit IL-6, IL-12, MCP-1 and TNF production when added simultaneously to activated monocytes whereas they enhance the production of these cytokines when they are delivered before the activating signals (16,36,50). When IL-4 was added simultaneously to TNF- $\alpha$ , it had a very low capacity to reduce the induction of tissue factor expression on the surface of endothelial cells (32). Conversely, a pre-treatment of the cells with IL-4 for 8 to 16 hr allowed a significant inhibition (48). In an elegant model of resistance to systemic *Pseudomonas aeruginosa* infection, Giampietri *et al.* (28) demonstrated that a 24 hr pre-treatment of mice with IL-4 was protective when high number of CFU were injected whereas when injected only 1 hr before the bacterial challenge with a lower number of CFU, IL-4 was deleterious. In the first case enhanced survival was associated with a reduced level of circulating TNF while in the later one reduced survival was associated with an enhanced level of circulating TNF. Another fascinating example of timing is provided by the effect of cortisol infusion in human volunteers. While an injection of LPS at the end of the cortisol infusion did not lead to detectable

circulating TNF, the same injection made 12 to 144 hr after the infusion led to far higher levels of TNF and IL-6 than those reached in the same volunteers who did not receive the cortisol pre-treatment (3).

### THE SEQUENCE OF CYTOKINE ACTION

Cytokines are the words of a universal language used by cells. As in any language, the order of the words influences the meaning of the sentence. Accordingly, the sequence of exposure to cytokines plays a key role in the nature of the signals delivered to the cells. For example, TNF and IFN- $\gamma$  used simultaneously have no significant effect on the production of NO by rat bone marrow-derived macrophages. In contrast, IFN- $\gamma$  primes the cells which then produce significant amounts of NO when exposed to TNF. Most interestingly, if the cells are first exposed to TNF, then 4 hrs later to IFN- $\gamma$  and after an additional 4 hrs finally exposed to TNF, they do not produce any NO (21). The same desensitization was observed with a pre-treatment with IL-4 or TGF- $\beta$  whereas IL-10 had no inhibitory activity in this model. A similar observation has been made when the LPS-induced production of IL-12p70 was investigated (33): cells pre-exposed to IFN- $\gamma$ , produced significant amounts of IL-12 whereas low or no production was obtained with cells pre-treated with either TNF or TNF + IFN- $\gamma$ .

### THE EXPERIMENTAL MODEL

We have studied *in vitro* the effect of IL-10 pre-treatment on the production of TNF and IL-6 by leukocytes upon stimulation by LPS. We reported that in the presence of IL-10, the prevention of monocyte adherence by red cells in the whole-blood assays or by cultures of peripheral blood mononuclear cells on Teflon<sup>®</sup>, allowed a higher cytokine production as compared to cells maintained in culture medium alone before the LPS activation. When the first step of the experiment was performed on plastic (i.e. with adherence of monocytes) the classical inhibitory activity of IL-10 was found (1). Altogether, these results indicate that IL-10-induced modulation of cytokine production depends on the *in vitro* experimental procedures. More recently, a similar "pro-inflammatory" activity of IL-10 was reported in human volunteers receiving an LPS injection (39). The use of different *in vivo* models may result in completely opposite conclusions. Indeed, in a model of immune complex-induced acute lung injury it was reported that the neutralization of IL-13 increased the

inflammatory process, suggesting that endogenous IL-13 restrained inflammation (41). In contrast, transgenic mice over-expressing IL-13 in the lungs showed an inflammatory mononuclear infiltrate, eosinophils around airways and in parenchyma, an airway epithelial hypertrophy, a goblet cell hyperplasia, a hyperproduction of mucus and a selective local production of the eotaxin chemokine (78). This last paper is reminiscent of the inflammatory role of IL-13 demonstrated in various models of asthma (73).

We already mentioned the protective role of IFN- $\gamma$  in a model of collagen-induced arthritis and the accelerated onset of the disease in IFN- $\gamma$ -KO mice (70). Billiau's group further demonstrated that this observation was only true when collagen was injected together with complete Freund adjuvant (CFA). Indeed, when incomplete Freund adjuvant was employed, the disease did not occur in the IFN- $\gamma$  receptor knock-out animals (49). The authors demonstrated that on one hand IFN- $\gamma$  induced pro-inflammatory cytokines such as TNF and IL-12, on the other hand, in the model using CFA (i.e. associating *Mycobacteria*), IFN- $\gamma$  had a beneficial role by restraining both the expansion of hematopoietic process and the number of macrophages, a major source of pro-inflammatory cytokines.

## IL-6, THE PARADIGM OF AMBIGUITY!

Acute phase proteins are essentially protective and limit the inflammatory process. They possess anti-protease and some scavenger activities. Accordingly, IL-6 can be considered as an anti-inflammatory cytokines thanks to its potency to induce the release of acute phase proteins by hepatocytes, including IL-1ra (27). It was also mentioned that IL-6 inhibited the release of IL-1 and TNF (61) and favored that of soluble TNF receptor (66). Accordingly, numerous experimental models, including systemic or local endotoxemia demonstrated the protective activity of IL-6 (75,76). However, in contrast, IL-6 can induce bone resorption (34), muscle atrophy (68), anemia (35) and can prime neutrophils for the production of PAF and superoxide anion (8,10). While IL-6 does not activate endothelial cells, it induces MCP-1, -3 and IL-8 production, STAT-3 activation, and ICAM-1 expression, in the presence of its soluble receptor which is naturally found in plasma (58). Deleterious activities of IL-6 *in vivo* have been suggested by experimental models of ischemia reperfusion and of lung injury performed in IL-6 knock-out mice which were shown to exhibit lower inflammatory responses (14,15).

## CONCLUSION

We have to admit that dogma often result from an oversimplification of the described phenomena. Accordingly, dogma are made to be broken! It appears that the inflammatory response is an extremely complex interplay of mediators whose exact contribution may depend on many influencing parameters. Finally, to add to the complexity, one should not forget that humans are not equal in terms of their inflammatory responses. The known genetic polymorphisms for many pro- as well as anti-inflammatory cytokines (17,54,69,72) are associated with the amplitude of the inflammatory process. In addition, another polymorphism exists in terms of target cell reactivity in response to cytokine signaling (5). This individual heterogeneity has also to be considered when addressing the inflammatory response.

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