The Pro-Inflammatory Cytokine Cascade

J.-M. Cavaillon and M. Adib-Conquy

Introduction

The production of pro-inflammatory cytokines is a prerequisite for initiating the anti-infectious process, whereas their exacerbated production during severe inflammation may contribute to deleterious consequences. The capacity of interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α) to induce inflammatory mediators contributes to their pro-inflammatory properties. Phospholipase, cyclooxygenase and lipoxygenase are activated by IL-1 and TNF-α leading to the release of prostaglandins, thromboxane, leukotrienes, and platelet activating factor (PAF). Free radicals (superoxide [O_2^-], nitric oxide [NO]), and proteolytic enzymes are other mediators produced by target cells in response to IL-1 and TNF-α. Other cytokines, including chemokines such as IL-8 or some T-cell derived cytokines, such as lymphotoxin-α are also involved in the cytokine cascade (Fig. 1). Different experimental approaches have demonstrated the contribution of the pro-inflammatory cytokines to the harmful effects observed in sepsis, trauma, burns, hemorrhage, severe surgery and other pathophysiological situations leading to systemic inflammatory response syndrome (SIRS). The injection of recombinant pro-inflammatory cytokines mimics some of the clinical parameters observed in SIRS patients and the use of anti-cytokine antibodies prevents most of the deleterious effects observed in animal models of SIRS.

Nature of the Stimuli Which Initiate the Cascade

When inflammation is initiated by an infectious process, the presence of microorganisms and their derived products (membrane compounds, released toxins, intra-cellular constituents following lysis) are potent activators of cytokine production. Macrophages are probably one of the major sources of cytokines. Among Gram-negative bacterial derived compounds, endotoxin or lipopolysaccharide (LPS) is a potent inducer of cytokines. During Gram-positive bacterial infection, membrane compounds such as peptidoglycan or lipoteichoic acid are strong inducers of macrophage-derived cytokines. In addition, exotoxins behave as superantigens and trigger the release of T-cell-derived cytokines. Other cells can contribute to the release of cytokines. For example, the beneficial TNF-α observed in experimental peritonitis has been demonstrated to be released by mast

J. C. Marshall et al. (eds.), *Immune Response in the Critically Ill*

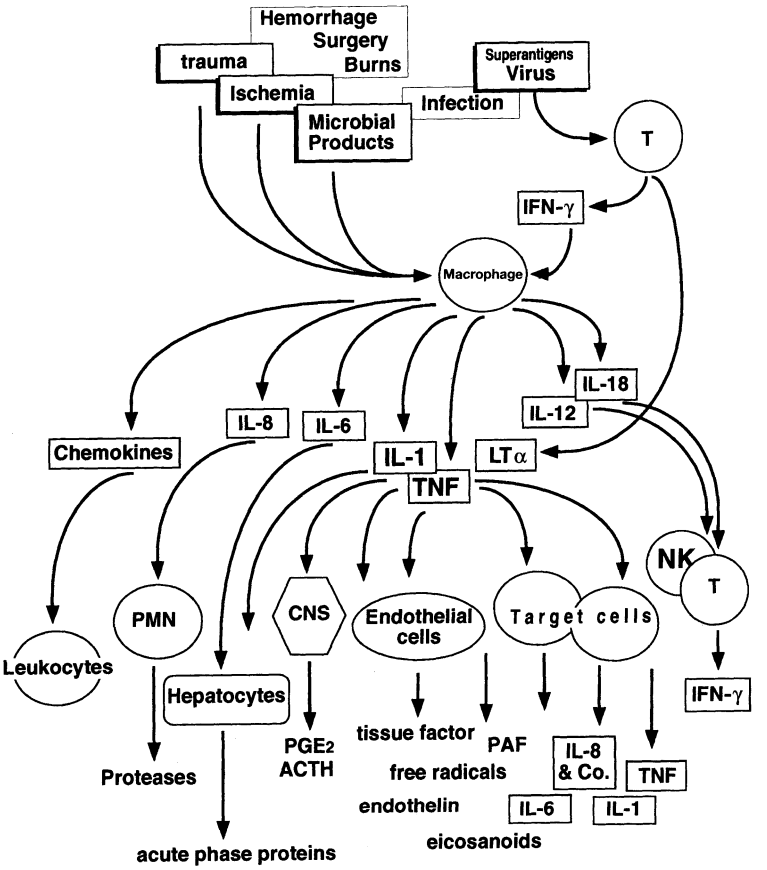

Fig. 1. Schematic representation of the pro-inflammatory cytokine cascade

cells [1]. So, in addition to the well known contribution of mast cells to inflammation observed in acquired immunity via the specific IgE antibodies, these cells play a central role during natural immunity by their capacity to release pro-inflammatory mediators in response to bacteria. Obviously, when the host is facing a stressful condition, many defense systems are activated, leading to the generation of newly synthesized or neo-generated compounds, as well as the release of pre-formed mediators. These factors have the capacity to modulate the generation of cytokines. Activation of the complement system and activation of the coagulation cascade lead to the appearance of factors (anaphylatoxins, thrombin, factor Xa) which can further enhance release of pro-inflammatory cytokines by activated macrophages. Once generated, cytokines possess the capacity of perpetuating their own production. In addition certain cytokines such as those generated by T lymphocytes (e.g., IL-3, granulocyte/macrophage colony-stimulating factor [GM-CSF]) and particularly Th1 cells (i.e., IL-2, IFN-γ) can amplify the release of the pro-inflammatory cytokines.

Bacterial derived products can also be triggering signals in pathophysiological situations that involve bacterial or endotoxin translocation. Hemorrhagic shock, burns or trauma can induce such translocation [2–4]. We have shown that in patients undergoing abdominal aortic surgery, which might be associated with mild gut ischemia, aortic clamping resulted in measurable amounts of circulating LPS and high levels of portal TNF-α suggesting local production by gut associated macrophages [5]. In an animal model, hemorrhage was also associated with local intestinal IL-6 production [6]. Other sites of production have been demonstrated during experimental models of hemorrhage, such as the lungs [7] and the peritoneum [8] where mononuclear phagocytes are a source of IL-1 and TNF-α. Very interestingly, it has been shown that resident mast cells within the tissue exposed to ischemia/reperfusion can degranulate and release pre-formed TNF-α [9]. In experimental liver ischemia and reperfusion experiments, reducing the production of IL-1 and TNF-α resulted in the reduction of polymorphonuclear leukocyte (PMN) infiltration and hepatic injury [10]. Ischemia is also associated with the local production of chemokines that contribute to the recruitment of inflammatory leukocytes [11]. *In vitro* experiments have demonstrated that hypoxia *per se* was capable of inducing the production of IL-1 and TNF-α by mononuclear cells [12]. Similarly, hypoxia induces the release of inflammatory cytokines by endothelial cells [13, 14]. Cardiopulmonary bypass (CPB) represents another inflammatory situation where the production of inflammatory cytokines occurs in various sites including peripheral blood as demonstrated by the presence of increased levels of cell-associated IL-1 in circulating monocytes [15]. The presence of cytokine transcripts in the skeletal muscle or the myocardium, and higher levels of TNF-α and IL-6 in coronary sinus blood than in arterial blood illustrates the various sites of production during CPB [16, 17]. Local production has been demonstrated within the site directly exposed to inflammation during acute pancreatitis [18], brain injury [19, 20], chest trauma [21] or laparotomy [22]. The compartmentalization of cytokine production was elegantly demonstrated in human unilateral pneumonia [23]. Bronchoalveolar lavage (BAL) levels of TNF, IL-1 and IL-6 and spontaneous production by alveolar macrophages were higher in the involved lung as compared to the non-involved lung.

Involvement of Pro-Inflammatory Cytokines in Inflammation

Activation of Endothelial Cells and Leukocyte Adherence to Endothelium

Endothelial cells are important target cells and actors during the inflammatory response. They constitute the interface between the injured tissues and the circulating leukocytes that need to be recruited. Endothelial cells are active producers of inflammatory mediators. In response to IL-1 and TNF-α, endothelial cells synthesize phospholipase and cyclooxygenase leading to the production of prostaglandins, express tissue factor enhancing the coagulation process, and release PAF, free radicals and a large panel of cytokines including IL-6 and IL-8. In addition, these cells express on their surface enhanced levels of adhesion molecules.

L- and P-selectins favor the capture of circulating leukocytes. The rolling of the cells onto the endothelium is mainly mediated by the E-selectins which together with the integrins lead to the firm adhesion of the leukocytes. Finally, integrins are involved in margination towards the tissues. Upon activation of endothelial cells by IL-1 or TNF-α, the expression of the E-selectin ELAM-1 (endothelial leukocyte adhesion molecule-1) is observed within one hour. Monocytes and neutrophils, harboring the counter-ligand Lewis X antigen, bind to the endothelium. The expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) occurs 4 h following IL-1 or TNF-α activation. *In vivo*, enhanced expression of these adhesion molecules has been demonstrated in various inflammatory processes. For example, augmented expression of ELAM-1 and ICAM-1 has been reported in the skin biopsies from atopic patients challenged locally with the corresponding allergen [24]. The counter-ligand for VCAM-1 is the VLA-4 molecule expressed on the surface of monocytes and lymphocytes. The counter-ligands for ICAM-1 are the LFA-1 (CD11a/CD18) and CR3 (CD11b/CD18) molecules found on the surface of monocytes and neutrophils. The activation of circulating cells leads to conformational changes of the LFA-1 and CR3 molecules that are necessary to favor a strong interaction with their ligands. Once leukocytes have adhered to endothelium, they migrate in response to chemoattractant signals locally delivered by chemokines. Blocking the chemokines by specific antibodies is associated with a lower recruitment of circulating cells and reduced inflammation [25]. Consequently, in infectious models, blocking chemokines is associated with impaired control of infection [26].

Cytokines and Coagulation

IL-1 and TNF are able to induce pro-coagulant activity on endothelial cells. Similar observations are reported with monocytes. This activity is a result of the induction of the membrane expression of tissue factor. Tissue factor combines with factor VII and initiates the coagulation cascade, activating factors X and IX. Injection of TNF in humans is associated with enhanced detection of factor X activated peptide and fragments 1 + 2 of thrombin [27]. In fact, TNF-α and IL-1 first activate fibrinolysis as assessed by enhanced levels of tissue plasminogen activator. An enhanced level of the inhibitor of tissue plasminogen activator occurs later and only then does thrombin become apparent. TNF-α and IL-1 activate coagulation via the induction of tissue factor, whereas IL-6 can also induce coagulation, independent of the induction of tissue factor. In contrast to IL-1 and TNF-α, IL-6 cannot induce fibrinolysis.

Induction of Lipid Mediators and Free Radicals

Many cells, in response to activation by IL-1 or TNF-α, release mediators derived from the metabolism of arachidonic acid either via the cyclooxygenase pathway, leading to the release of prostaglandins and thromboxanes, or via the lipoxygen-

ase pathway leading to the release of leukotrienes. Arachidonic acid itself is generated from membrane phospholipids following the action of phospholipase A2. Neosynthesis of phospholipase A2 and inducible cyclooxygenase (COX2) occurs following the action of IL-1 or TNF-α. Prostaglandins are involved in smooth muscle contraction, mucosal edema, increased vascular permeability, cellular infiltration and mucus secretion. In addition, eicosanoids can modulate the production of pro-inflammatory cytokines: For example, prostaglandin E2 (PGE2) inhibits TNF-α production, but has no significant effect on IL-1, and enhances IL-6 production, while thromboxane A2 favors TNF-α and IL-1β production. Many activities generated by IL-1 and TNF can be suppressed by the use of cyclooxygenase inhibitors such as aspirin or ibuprofen, illustrating the contribution of PGE2 to the activities initiated by IL-1 and/or TNF-α. The action of phospholipase A2 on phosphatidylcholine generates arachidonic acid and lyso-PAF. The latter will be transformed by acetyltransferase to PAF. This factor, known to activate and aggregate platelets, is also responsible for the release of vaso-active mediators, resulting in increased permeability, vasoconstriction and bronchoconstriction.

Among the mediators induced by IL-1 and/or TNF, endothelin also acts on endothelial cells leading to vasoconstriction and increased blood pressure. Four different endothelins have been described. They are short 21 amino acid peptides derived from a 39 amino acid precursor. Endothelin acts via a 7 transmembrane domain receptor coupled to G protein.

Free radicals are also induced upon activation of the oxidative burst by IL-1 and TNF-α. Superoxide anion (O_2^-) generated upon the action of a membrane enzyme NADPH oxidase, has an anti-microbial activity but can also be toxic to local cells. It induces the peroxidation of unsaturated fatty acids leading to an alteration of membrane fluidity and permeability, as well as to the oxidation of amino acids resulting in an alteration of the proteins. Activation of the manganese superoxide dismutase by IL-1 and TNF-α generates hydrogen peroxide (H_2O_2). NO is generated from L-arginine through the action of NO synthase (NOS). Three NOS have been described – two are constitutive (NOS-1 involved in neuronal transmission and NOS-3 which contributes to the vasodilatation at homeostasis) and one is inducible (NOS-2) and produced by many cell types upon activation by IL-1 and TNF-α as well as by interferon-γ (IFN-γ) and migration inhibitory factor (MIF). The toxicity of NO is a result of its ability to inhibit glycolysis, the Krebs cycle, mitochondrial respiration, and DNA synthesis. Very unstable, NO is rapidly transformed to nitrite (NO_2^-) in the presence of H_2O and to nitrate (NO_3^-) by the effect of oxyhemoglobin.

Cytokines and Catabolism

IL-1 was previously known as catabolin and as osteoclast activating factor, a reflection of its ability to generate degradation of cartilage and to induce bone resorption. IL-6 is another cytokine which has been shown to be involved in osteolysis, particularly during the post-menopausal period. Muscle proteolysis is

also observed during inflammation. The release of amino acids from muscle further enhances the pool of free amino acids available for the neosynthesis of inflammatory proteins. TNF and to a lesser extent IL-1, have this capacity when acting on muscles. Furthermore, the activation of neutrophils leads to the degranulation of these cells and thus to the release of proteases such as cathepsin and collagenase which can degrade the extracellular matrix, as well as other cellular constituents.

The Main Pro-Inflammatory Cytokines

Interleukin-1

Injection of IL-1 into animals results in hypotension, increased cardiac output and heart rate, leukopenia, thrombocytopenia, hemorrhage, pulmonary edema [28] associated with pulmonary vascular endothelial injury [29], lacticacidemia, hypoaminoacidemia and histopathological lesions in the adrenal cortex [30]. Cyclooxygenase inhibition greatly prevents these effects. IL-1 receptor antagonist (IL-1ra) is a natural IL-1 inhibitor. Early treatment with IL-1ra reduced mortality from endotoxic shock [31, 32], prevented *Staphylococcus epidermidis*-induced hypotension [33], improved survival and hemodynamic performance in *Escherichia coli* septic shock [34] and, depending on the dosage, either reduced or enhanced lethality in a model of *Klebsiella pneumoniae* infection of new born rats [35]. In agreement with these observations, IL-1ra-deficient mice are more susceptible than controls to lethal endotoxemia [36]. IL-1 converting enzyme (ICE), or caspase-1, is an enzyme required for the maturation of the 30 kDa biologically inactive IL-1β precursor to the mature 17 kDa active form of IL-1β. Survival to a lethal dose of endotoxin reached 70% among ICE-deficient animals [37] and these ICE-deficient animals showed a 50% decrease in cellular infiltrate during zymosan-induced peritonitis [38]. On the other hand IL-1β deficient mice were normally sensitive to the lethal effect of LPS [39]. The latter result suggests that, in mice, IL-1α can fulfill the role of IL-1β.

Tumor Necrosis Factor-α

TNF-α toxicity includes hemodynamic instability, fever, diarrhea, metabolic acidosis, capillary leak syndrome, activation of disseminated intravascular coagulation (DIC), late hypoglycemia, induction of a catabolic state, neurotoxicity, cachexia, organ edema, renal and hematological disorders, and acute pulmonary dysfunction, all phenomena associated with sepsis syndrome, systemic inflammatory response syndrome and the genesis of multiple organ failure (MOF) [40–44]. In a cancer patient receiving a high dose of recombinant TNF-α, a systemic inflammatory response syndrome was reported with marked hypotension, extreme generalized capillary leak syndrome and pulmonary function deterioration [45] while hepatic toxicity had been previously reported [46]. In addi-

tion, together with IL-1, TNF-α efficiently induced on endothelial cells the expression of adhesion molecules, an event which favors organ infiltration by leukocytes. Its lethal effect was synergistically enhanced by IL-1 [28], IFN-γ [47] and LPS itself [48]. Furthermore, its toxicity was influenced by circadian rhythms [49] and the route of infusion: Portal infusion of TNF-α led to high mortality, renal necrosis, and gut mucosal destruction, an effect not observed after systemic injection [50]. Anti-TNF treatments have been shown for more than a decade to be highly effective in protecting animals against endotoxic shock [51] and lethal bacteremia [52]. Such treatments also protect against pulmonary microvascular injury after intestinal ischemia injury that is associated with endotoxin translocation [53]. The soluble forms of the TNF-α receptors are natural inhibitors capable of limiting TNF-α bioactivity. Their injection into ammal models of sepsis has also been shown to be essentially protective [54–56]. Mice rendered deficient for the p55 TNF-α receptor were resistant to endotoxin in the galactosamine model, whereas high doses of LPS in the absence of galactosamine led to a lethality similar to that observed in wild-type animals; similar findings were obtained with p75 TNF receptor deficient mice [57, 58] and TNF-α/lymphotoxin-α deficient mice [59]. In contrast, over-expression of TNF in transgenic animals is associated with a severe inflammatory process localized in the over-expressing tissues, ending in cardiac failure [60], degeneration of the central nervous system [61] or erosive arthritis [62].

Interleukin-12

Among the adverse effects of IL-12, hepato- and splenomegaly, leukopenia, anemia and myelodepression have been reported [63]. These phenomena are largely IFN-γ-dependent since they have not been reported to occur in IFN-γ receptor deficient mice. Hepatomegaly is associated with infiltration of activated macrophages and natural killer (NK) cells, and single-cell necrosis. In contrast, pulmonary edema and interstitial macrophage infiltration generated by IL-12 injection has been shown to be IFN-γ-independent. In a bacillus Calmette-Guérin (BCG)-primed model of LPS-induced shock and lethality, anti-IL-12 antibodies were shown to protect mice if injected before endotoxin [64]. The protection was associated with decreased IFN-γ production.

Interferon-γ

Side-effects of IFN-γ include tachychardia, myalgia, malaise, leukopenia, and weakness. Furthermore, its synergy with the detrimental activities of LPS have been clearly established: IFN-γ enhanced LPS-induced mortality and increased levels of LPS-induced circulating TNF-α [65]. Consequently, anti-IFN-γ antibodies protected against LPS- and *E. coli*-induced mortality [65, 66]. In addition, IFN-γ was shown to be a mediator of TNF-α-induced lethality. Sublethal doses of TNF-α and IFN-γ, when injected together, led to 100% lethality in mice, and anti-

IFN-γ antibodies protected against one to four LD100 of TNF-α [47]. Mice lacking IFN-γ receptors have been shown to be resistant to LPS challenge after priming with BCG [67] or treatment with galactosamine [68].

Leukemia Inhibitory Factor (LIF) and Oncostatin M (OSM)

LIF and OSM belong to the IL-6 superfamily, sharing the gp130 chain of the receptor. However, while IL-6 and IL-11 possess certain anti-inflammatory properties (see below), LIF and OSM can be considered as pro-inflammatory cytokines. Indeed, LIF is involved in the pathogenesis of inflammation and sepsis syndrome [69]. Produced upon activation by LPS and TNF, LIF can itself induce the release of other cytokines, including IL-1, IL-6 and IL-8, by various cell types. Passive immunization against LIF in mice challenged with intraperitoneal administration of endotoxin protects them from the lethal effects and blocked increases in serum levels of IL-1 and IL-6 [70]. Levels of plasma LIF, ciliary neurotrophic factor (CNTF, another member of the IL-6 family), and OSM are elevated in septic patients [71]. Subcutaneous injection of OSM in mice causes an acute inflammatory reaction [72]. OSM favors PMN adhesion to endothelial cells and transmigration via its capacity to enhance the expression of P- and E-selectin, ICAM-1 and VCAM-1. Furthermore, OSM induces the release of IL-6 and ENA78 (an α-chemokine), but not that of IL-8.

Macrophage Migration Inhibitory Factor

Recent investigations on pituitary-derived factors resulted in the rediscovery of an old cytokine named MIF. Bernhagen et al. [73] reported that injection of MIF together with one LD40 of LPS greatly potentiated lethality, and anti-MIF antibodies fully protected against one LD50 of LPS. As previously mentioned, MIF acts to counter-regulate the inhibitory effects of glucocorticoids on inflammatory cytokine production (Fig. 2). Interestingly, it was recently shown that MIF is expressed constitutively in many tissues including lung, liver, kidney, spleen, adrenal gland, and skin. MIF exists as a preformed cytokine which is rapidly released following LPS injection [74].

Interleukin-8 and the Chemokines

Sepsis and SIRS are often associated with organ dysfunction, a reflection of the inflammatory process occurring in the tissues. One of the major features of this phenomenon is the recruitment of inflammatory leukocytes, the adherence of circulating cells to the endothelium and their response to the locally produced chemokines. Endothelial cells are highly responsive to IL-1 and TNF, in terms of adhesion molecule and tissue factor expression, as well as cytokine production.

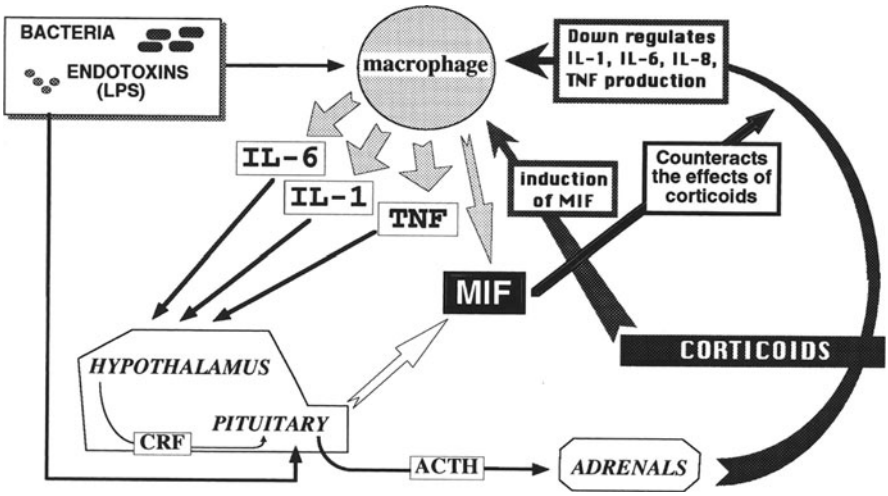

Fig. 2. Schematic representation of the regulatory loop involving down-regulation of pro-inflammatory cytokine production by glucocorticoids and its regulation by macrophage migration inhibitory factor (MIF) which behaves as a pro-inflammatory cytokine

Endothelial cell wall integrity is also perturbed by these cytokines as illustrated by the absence of endothelial damage in IL-1 receptor type I deficient mice [75]. Mice have been developed lacking molecules normally expressed on circulating leukocytes (e.g., L-selectin, CD62L) or on endothelial cells (e.g., ICAM-1). These adherence molecules are involved in the attachment of neutrophils, lymphocytes or monocytes to the endothelium [76, 77]. These deficient mice are significantly resistant to LPS-induced toxic shock lethality. Once immobilized on endothelium, leukocytes will migrate towards the tissues in response to IL-8 and the other chemokines. Thus, these chemokines favor the inflammatory cell infiltrate which contributes to the loss of tissue integrity. For example, it has been reported that neutralization of IL-8 profoundly inhibits neutrophil recruitment in an endotoxin-induced rabbit model of pleurisy, indicating that IL-8 is a major chemotactic factor in this model of acute inflammation [78]. During sepsis a large amount of IL-8 is detectable within the blood compartment, not only as a free cytokine [79] but also as a cell-associated form [80]. This first encounter of neutrophils with IL-8 led to their desensitization to further signals delivered locally by IL-8. Thus, the presence of IL-8 in the vascular space may well be a mechanism for limiting neutrophil accumulation at extracellular sites as illustrated by the defect in neutrophil migration during sepsis or endotoxemia [81–83]. Similarly, while monocyte-chemoattractant protein-1 (MCP-1) contributes to the recruitment of inflammatory macrophages within the tissues, neutralization of MCP-1 by specific antibodies before LPS administration resulted in a striking increase in mortality and injection of MCP-1 was protective [84].

The Beneficial Effects of IL-6 and IL-11

Many investigators have demonstrated that levels of circulating IL-6 correlate with severity of sepsis and may predict outcome [85–88]. Although IL-6 is often considered to be an inflammatory cytokine, most of its activities are associated with a negative control of inflammation. For example, IL-6 induces the release of IL-1ra and soluble TNF receptors [89]. Its most potent anti-inflammatory activity is linked to its capacity to induce the release of acute-phase proteins. In this context, it is interesting to note that IL-1ra has recently been identified as a product of hepatocytes, and regulated by pro-inflammatory cytokines like acute-phase proteins [90]. Furthermore, some acute-phase proteins such as the C-reactive protein (CRP), α1-anti-trypsin, and α1-acid glycoprotein, induce IL-1ra [91]. Reports have demonstrated that these acute-phase proteins can limit the inflammatory process [92], protect against meningococcal endotoxin [93], or even inhibit a lethal response to TNF [94, 95]. These results may explain why IL-6 has been shown to be protective in infectious and in septic shock models [96–98]. Nevertheless, IL-6 does possess some deleterious and pro-inflammatory effects which will be detailed below [99–102].

IL-11 belongs to the IL-6 superfamily, sharing the gp130 chain of the receptors. Although IL-11 stimulated the production of several major acute phase proteins by hepatoma cells, circulating IL-11 did not significantly participate in the production of acute-phase proteins by the liver [103]. One of the major beneficial effects of IL-11 is related to its healing activity on the intestinal tract. For example, chemotherapy and radiation both damage the small intestine mucosal barrier and lead to the entry of gastrointestinal organisms into the blood. In this lethal model, IL-11 was able to protect 80% of the animals [104]. Beneficial properties of IL-11 have also been demonstrated in a rat neonatal infectious model with group B streptococci. Prophylactic use of IL-11 enhanced the survival in this model in association with an increased number of platelets [105].

Are IL-1 and TNF the Main Directors of the Cascade?

In many pathophysiological situations, it has been reported that the production and the presence of pro-inflammatory cytokines correlates with the severity of the disease. For example, during cardiac surgery and in patients undergoing CPB, it was shown that TNF-α levels correlated with the duration of bypass and were associated with clinical complications which led to the development of SIRS/multiple organ dysfunction syndrome (MODS) [106]. More convincing is the direct demonstration that blockade of pro-inflammatory cytokines ameliorates the pathophysiological aftermath and improves survival in acute inflammatory diseases. Antibodies against TNF-α illustrate its role as a main mediator involved in the progression from local inflammation to a host-wide syndrome of organ injury. Thus anti-TNF-α antibodies were beneficial in various animal models of acute pancreatitis [107, 108], splanchnic artery occlusion [109], allograft rejection [110] or IL-2 toxicity [111]. Anti-TNF treatments reduced pulmonary injury fol-

lowing lower torso [112], hepatic [113], or intestinal [53] ischemia/reperfusion as assessed by reduced hemorrhage, edema, PMN sequestration, capillary leak and rise of leukotriene levels in BAL. Similarly, in hemorrhagic shock models, the use of anti-TNF-α demonstrated the contribution of this cytokine to the hemodynamic alterations, organ injury and mortality [114, 115]. In a model of zymosan-induced MODS Goris' group showed that treatment with a monoclonal antibody against TNF-α improved survival [116]. However, in humans, the therapeutic use of various anti-TNF-α approaches in sepsis has so far proven ineffective. In contrast, the use of anti-TNF-α antibodies has been shown to be particularly effective in the treatment of two chronic inflammatory diseases – rheumatoid arthritis [117] and Crohn's disease [118].

While these data indicate that TNF-α plays a major role in various acute inflammatory diseases, none fully demonstrate whether TNF is necessary for the deleterious effects associated with severe diseases. The use of TNF-deficient animals can help to answer this question. Interestingly, we demonstrated that in TNF-α/lymphotoxin-α deficient animals, LPS-induced lethality was similar to that of control animals [59], suggesting that death following endotoxin-induced shock could occur in the absence of TNF. Furthermore, we showed that 90 minutes after LPS injection the levels of circulating IL-6 in control and knock-out animals were similar, while after 3 hours, higher levels were observed in normal mice. These results indicate that TNF is not necessary for IL-6 production although its presence further enhances it. These results contrast with experiments performed with anti-TNF-α treatments which led to a significant decrease of IL-1, IL-6 and IL-8 at any time after bacterial injection [119]. On the other hand, survival can occur even with the presence of abundant circulating levels of TNF-α. In a rabbit model of endotoxin shock we have shown that hemofiltration coupled with resin adsorption significantly protected the animals from death while the levels of circulating bioactive TNF-α remained unchanged (Tetta et al., unpublished data).

Using IL-1ra to counteract the effects of IL-1, the contribution of IL-1 to various acute inflammatory diseases including pancreatitis [120], acute respiratory distress syndrome (ARDS) [121], and immune complex-induced colitis [122] or lung injury [123] has been similarly demonstrated. IL-1ra is capable of preventing LPS-induced lethality and most of the endotoxin-induced injury. The inhibition of inflammation by IL-1ra is associated with a decrease in detectable IL-1 and TNF-α as shown in the lungs of mice following hemorrhage and resuscitation [124]. While the decrease of inflammatory cytokines reflects reduction of the inflammatory process, it is also the direct consequence of a reduced production of IL-1 and TNF-α by activated phagocytes [125]. These observations further illustrate the auto-regulatory loops between IL-1 and TNF-α; each can induce the other, as well as itself.

Signaling by Pro-Inflammatory Cytokine

The Nuclear Factor-κB (NF-κB) Pathway

The inducible transcription factor NF-κB plays a major role in intracellular signaling, during inflammatory processes induced by stress, mitogens or cytokines. Indeed, this is one of the main nuclear factors that regulates the transcription of numerous genes, including cytokines and growth factors (especially pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8), cytokine receptors, stress proteins and leukocyte adhesion molecules [126, 127]. The NF-κB family is composed of various members, p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB and c-Rel, which can form homo- and heterodimers [128]. In most cells, the complex that is commonly found is the p50p65 heterodimer which is a potent transactivator, while it is generally believed that the p50p50 homodimer is not [129]. NF-κB is regulated by a cytoplasmic inhibitor: IκB. This protein also is member of a large family that includes IκBα, IκBβ, IκBγ, IκBε and Bcl-3. All possess multiple regions of homology known as the ankyrin-repeat motifs. These motifs are also present in the precursors of p50 and p52, p105 and p100 respectively, which also behave as NF-κB inhibitors. In unstimulated cells, NF-κB is retained in the cytoplasm by IκB as an inactive complex. Upon stimulation, IκB is phosphorylated on serines 32 and 36, leading to its subsequent ubiquitination and its degradation by the 26S proteasome pathway. TNF-α and IL-1 are potent activators of NF-κB. As represented in Figure 3, the binding of TNF-α or IL-1 to their receptors recruits adaptator molecules which lead to the activation of an NF-κB-inducing kinase (NIK) [130]. The signaling by the TNFR1 involves a TNF receptor-associated death domain protein (TRADD) [131]. Death domains mediate protein-protein

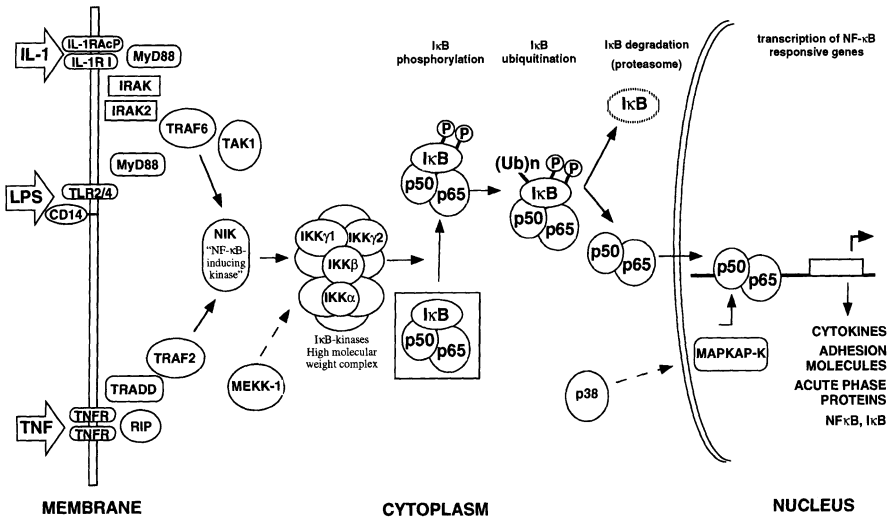

Fig. 3. Schematic cascade of NF-κB activation by TNF-α and IL-1. See text for abbreviations

interactions; TRADD can bind to the TNF receptor-associated factor-2 (TRAF2) and to receptor interacting protein (RIP), another death domain-containing kinase [132, 133]. IL-1 signaling also uses many receptor-associated proteins. The IL-1 receptor I associates with the IL-1 receptor accessory protein (IL-1RAcP) [134], and MyD88 a death domain-containing protein. MyD88 can interact with IL-1 receptor associated kinase (IRAK) that, via TRAF6, activates NIK, a MEKK-1 related kinase [135]. MEKK-1 is a mitogen-activated protein kinase (MAPK) kinase kinase implicated in one of the MAPK cascades (see the next section). The data about the contribution of this cascade to NF-κB activation remains controversial. Indeed, recombinant MEKK-1 has been shown to phosphorylate IκB *in vitro* [136] and its over expression in a fibroblastic cell line could induce NF-κB activation during TNF stimulation through the degradation of IκB [137]. However, another study showed that overexpression of inactive MEKK-1 has no inhibitory effect on NF-κB activation via TRAF2, suggesting that it does not play a role in the activation of this transcription factor [138]. Thus, it would appear that NIK, rather than MEKK-1 itself, takes part in NF-κB activation. Furthermore, NIK seems to be the convergence point of the TNF-α and IL-1-mediated NF-κB activation, since mutant forms of NIK block the signaling from the receptors of these cytokines [130]. The final step of the kinase cascade leads to the activation of protein kinases that phosphorylate IκB. These IκB kinases (IKK) are associated with a high-molecular weight cytoplasmic complex [139–141]. Overexpression of NIK, but also MEKK-1 has been shown to phosphorylate and activate IKKα and β. Finally, after the degradation of IκB, the NF-κB dimer can translocate into the nucleus, bind to DNA and activate the transcription of target genes.

The Mitogen-Activated Protein Kinases

The MAPK cascades are the other intracellular signaling pathway activated during the inflammatory process and they also lead to the activation of numerous transcription factors. Three MAPK cascades have been described to date – the extracellular signal-regulated kinases (ERK), the c-jun N terminal kinase/stress-activated protein kinase (JNK/SAPK) and the p38 pathways (Fig. 4). The first identified cascade was that of ERK. The activation of ERK1 and 2, also known as p44 and p42, is triggered by mitogens and growth factors, while the two other cascades are strongly activated by IL-1, TNF-α, LPS and cell stress [142, 143]. c-jun is a component of the activator protein(AP)-1 transcription factor and the JNK cascade leads to its phosphorylation and an enhancement of its capacity to activate transcription. The JNK pathway contains a MAPK kinase kinase, MEKK-1, which has been shown in some experiments to take part in the activation of NF-κB (see above). The p38 kinase is the last MAPK described to date and it has been implicated in the activation of various transcription factors and some reports suggest that it can play a role in the activation of NF-κB. Indeed, it has been shown that a specific inhibitor of p38 (SB203580) prevented the expression of a reporter gene under the control of NF-κB [144]. However, this was not due to an inhibition of the binding of NF-κB to DNA. Thus, p38 does not seem to regulate IκB phosphor-

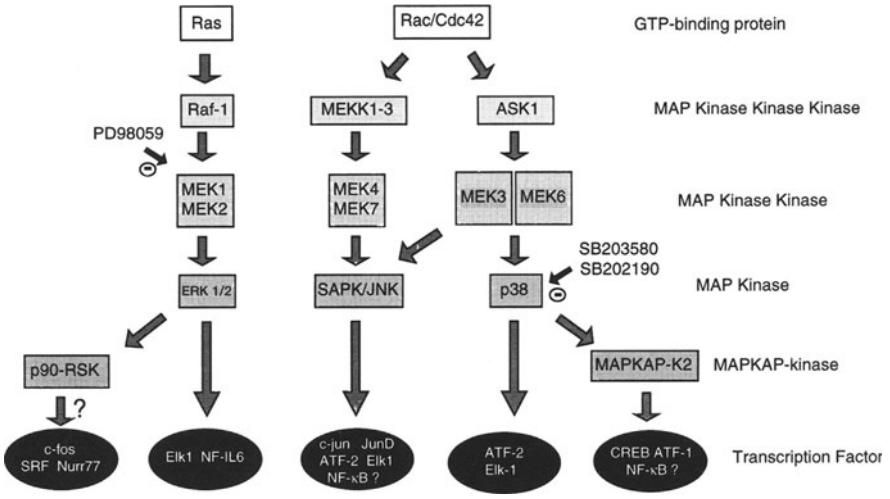

Fig. 4. Schematic representation of the three MAP kinase pathways (ERK, SAPK/JNK and p38). The ERK pathway is triggered by mitogens and growth factors while SAPK/JNK and p38 are strongly activated by endotoxin and also by cytokines such as TNF or IL-1

ylation, but most probably modulates the transactivation capacity of NF-κB via MAPKAP-kinases that in turn phosphorylate the p65 subunit. TNF and IL-1 contribute to the activation of JNK and p38 MAPK, but the molecular mechanisms between the receptors and these kinases are not completely understood. For TNF, it has been shown that TRADD, TRAF2 and RIP are implicated in the signaling leading to JNK and p38 activation [138, 145]. Furthermore, another kinase, the germinal center kinase (GCK), has been shown to interact with TRAF2 and MEKK-1 and thus could be the link between the events taking place at the receptor level and the MAPK kinase kinase [145]. For IL-1, MyD88 and IRAK are also needed for the signaling. IRAK-deficient mice showed reduced IL-1-mediated JNK and p38 activation [146]. Similarly, overexpression of MyD88 induced the activation of JNK and NF-κB while mutant forms of MyD88 inhibited their activation [135].

Inflammatory Stimuli-Induced Immune Suppression Limits the Pro-Inflammatory Cytokine Cascade

Immune depression is associated with numerous stressful conditions. Many immune responses such as delayed-type hypersensitivity, lymphoproliferative responses, NK activity, and cytokine production are diminished. Immunosuppression has often been associated with increased production of IL-10 and transforming growth factor-β (TGF-β) and levels of circulating IL-10 [147] and TGF-β [148] are increased in septic patients. Plasma IL-10 may contribute to the cell deactivation observed in septic and trauma patients [149, 150]. A similar hypothe-

sis has been proposed for TGF-β [151–153]. In addition, in humans, the use of many drugs may further modify cell reactivity [154–156].

Reduced *ex Vivo* Cytokine Production in Critically Ill Patients

Sepsis syndrome, surgery, trauma, hemorrhage and thermal injury are associated with an augmented *in vivo* production of pro- and anti-inflammatory cytokines as assessed by their increased levels in the blood stream. Paradoxically, the capacity of circulating leukocytes from these patients to produce cytokines is reduced when compared to cells from healthy controls. Although the hyporeactivity of the cells observed in septic patients has been associated with endotoxin tolerance [157], this phenomenon is specific neither for endotoxin [158] nor for septic patients. Hyporesponsiveness of circulating leukocytes and low cytokine production have been associated with immune depression observed in these patients.

Monocyte reactivity to LPS stimulation has been particularly studied in isolated monocytes and in whole-blood assays. Monocytes from septic patients show a diminished capacity to release TNF-α, IL-1α, IL-1β, IL-6, IL-10 and IL-12 [159–165] whereas this was not the case for IL-1ra [162]. Reduced cytokine production has also been observed with other stimuli such as silica, staphylococcal enterotoxin B (SEB), killed *Streptococcus* and *Staphylococcus* [165–168]. Similarly, in patients undergoing abdominal aortic surgery [169], cardiac surgery associated with CPB [170], or trauma [171], monocyte-derived cytokine production was significantly altered.

Similar hyporeactivity has been reported when studying neutrophils. In septic patients the production of IL-1β, soluble IL-1ra and IL-8 by LPS-activated neutrophils is lower than in healthy controls [157, 172].

Indeed, the very first observation of the hyporeactivity of circulating leukocytes was demonstrated with peripheral blood lymphocytes. In this initial study, Wood et al. [173] reported decreased IL-2 production upon phytohemaglutinin (PHA) stimulation in major burn patients, with a more severe depression during the septic episodes. In patients undergoing surgery with or without CPB, hemorrhage and in trauma patients, reduced mitogen-induced IL-2 has been documented [171, 174–177]. The defect in IL-2 production was previously shown not to be related to a different $CD4^+/CD8^+$ ratio [174]. However, the different results obtained when different cell activators are used suggests that either the proportion of certain T-cell subsets may be modified in critically ill patients, or an alteration in cell reactivity may only occur among certain T-cell subsets [176]. Furthermore, certain monocyte subsets, particularly the human leukocyte antigen (HLA) DR^+ cells, have a modified frequency in injured or septic patients [178], and may, as accessory cells, contribute to the different observed T cell reactivity. IL-2 production varies depending on the animal experimental models. Concanavalin (ConA)-induced IL-2 production by spleen cells was unaffected in endotoxemia [179] and reduced in trauma, hemorrhage, peritonitis, and thermal injury models [177, 180–183]. However, one should be very careful in extrapolating these data ob-

tained from animal models to the human situation. Mouse studies use spleen cells whereas human studies usually involve peripheral blood leukocytes. Circulating cells and cells within tissues are likely not affected similarly by the various stressful situations. IFN-γ *ex vivo* production was also reported to be altered in burns [183], trauma [171], hemorrhage [177], CPB [184] and in sepsis [165].

IL-2 and IFN-γ are both Th1 cytokines, therefore, it was of interest to know whether the production of Th2 cytokines was also modified during stressful situations. In sepsis, ConA-induced IL-2, IL-5, and IL-10 production by peripheral blood mononuclear cells was diminished, whereas this was not the case when PHA and anti-CD3 were employed as activation stimuli (Muret et al., unpublished data). In contrast, Miller-Graziano et al. [185] reported that PHA- and (anti-CD3 + anti-CD4)-induced IL-10 production were depressed in trauma patients. In animal models, the results depend on the insult and on the study. In experimental peritonitis following cecal ligature and puncture (CLP), ConA-induced IL-10 production by splenocytes was either enhanced [181] or reduced [186], and in models of hemorrhage, therma injury, and trauma, ConA-induced IL-10 production was enhanced [149, 177, 182, 183, 186].

In a mouse model of severe injury (fracture + hemorrhage) it was reported that splenocytes produce more IL-5 upon stimulation by ConA, together with an increase in IL-4 and IL-10, wheareas IL-2 production is decreased [182]. This animal model led Mack et al. [182] to postulate that there was a shift to a Th2-type splenocyte cytokine response after injury.

Reversibility of Immune Depression

The enhanced susceptibility of injured patients to infection is well known and associated with a depressed immune system. Attempts to restore immune cell reactivity have been made using IFN-γ. In mice, IFN-γ attenuated hemorrhage-induced suppression of macrophage and splenocyte function and decreased susceptibility to sepsis [187]. In humans, while the results did not show definite beneficial effects in severe surgery patients [188], IFN-γ restored monocyte reactivity and enhanced HLA DR^+ frequency [189]. Other cytokines such as IL-2, IL-12, and GM-CSF could be considered to restore immune cell activity. IL-2 enhances the survival of mice in a burn and sepsis model and its activity was enhanced by the addition of lymphokine-activated killer cells [190]. IL-12 and GM-CSF were capable of reversing the LPS-induced desensitization *in vitro* as well as *in vivo* [191, 192]. Interestingly, other natural mediators, notably growth hormone [193] and prolactin [194] are capable of abrogating the immune defects associated with stressful conditions and of protecting against sepsis.

A Too Simplistic Dichotomy

The events occurring during inflammation are not as simplistic as just an interplay between pro- and anti-inflammatory actors. Indeed, the situation is far more

complex. First, the genetic background contributes to the heterogeneity of the inflammatory response in humans. Genetic polymorphisms have been reported for many pro- as well as anti-inflammatory cytokines [195–198], and, in addition, another polymorphism exists in terms of target cell reactivity in response to cytokine signaling [199]. While an excess of pro-inflammatory cytokines may be deleterious and even kill when used in animal models, these same cytokines are essential in the initiation of the anti-infectious response. More recently, their anti-inflammatory potential has even been outlined. For example, in response to an injection of myelin oligodendrocyte glycoprotein, a model which mimics human systemic sclerosis, TNF deficient mice display a more severe auto-immune mediated demyelination than their wild type counterparts [200]. In *in vitro* models, when delivered early enough to the cells, TNF can prevent the INF-γ-primed production of NO [201] or IL-12 [202]. The amount of the delivered cytokine may also influence its property. For example, while low concentrations of IL-12 exacerbated the disease in an experimental model of arthritis, IL-12 behaved as an anti-inflammatory cytokine when delivered at higher concentrations [203]. Surprisingly, INF-γ could inhibit the production of LPS-induced chemokines MIP-1α and MIP-1β to the same extent as IL-10 and TGF-β [204].

IL-4, IL-10, IL-13, IFN-α and TGF-β are considered as anti-inflammatory cytokines because of their capacity to inhibit the release of pro-inflammatory cytokines, to induce the production of IL-1ra and the release of soluble TNF receptor, and to limit some of the pro-inflammatory activities of IL-1 and TNF. However, many available examples illustrate that the pro- or anti-inflammatory properties of these cytokines may depend on the nature of the target cell, the nature of the stimuli the cell has encountered, the sequence of the events and the nature of the environmental factors.

While IL-4 inhibits the IL-1- or TNF-induced expression of ICAM-1 and ELAM-1 on the surface of endothelial cells, it favors the expression of VCAM-1, allowing the adherence of basophils and eosinophils [205]. Also, IL-4 inhibits the LPS-induced production of IL-8 by macrophages, but amplifies that of endothelial cells [206]. Similarly, while IL-13 diminishes chemokine production in activated macrophages, it induces the synthesis of MCP-1 in endothelial cells [207]. Whether a mediator exerts an inhibiting or, on the contrary, an enhancing property 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 [208–210]. IL-4, which can block TNF production by LPS-activated freshly isolated monocytes, is unable to inhibit the TNF production by 7-day cultured monocytes stimulated by LPS but can block the IL-1 production [211]. The nature of the activator results in a different profile; IL-4 was shown not to interfere with the production of RANTES (regulated on activation, normal T expressed and secreted) by human monocytes activated by IFN-γ whereas it amplified it when the cells were activated by TNF [212]. Similarly, IL-13 inhibits TNF and IL-8 production in human whole blood assays in the presence of LPS while it does not modify the TNF production when heat killed streptococci are used as activators [213].

The nature of the responding cell may also influence the quality of the modulation of the response by TGF-β. While TGF-β1 limited the production of IL-1α and IL-8 in macrophages, it induced their production in epithelial cells [214].

IL-10 which is undoubtely an anti-inflammatory cytokine, has been shown in various models to behave differently. IL-10 may well have some pro-inflammatory properties. Evidence from *in vivo* work (graft rejection [215], ocular inflammation [216], auto-immune diseases [217], anti-tumor activity [218]) suggests that in some circumstances, IL-10 may also behave as an immunostimulatory and/or pro-inflammatory cytokine. Injection of an adenocarcinoma expressing IL-10 gene results in a far more severe local inflammation than the non-transfected adenocarcinoma, as evaluated in terms of the presence of MCP-1 and inducible NOS, of leukocyte infiltrate and expression of adherence molecules [219]. Some *in vitro* reports have shown that IL-10 could also favor certain aspects of the inflammatory response. Thus, IL-10 induced E-selectin expression on small and large blood-vessel endothelial cells [220]. IL-10 may behave differently depending on the nature of the target cells. For example, a consistent, or even enhanced, production of IL-8 has been reported by dendritic cells [221] and endothelial cells [206]. While IL-10 could perfectly repress the production of IL-8 by LPS-activated blood neutrophils, such inhibition could not be obtained when neutrophils were derived from the sputum of patients with chronic bronchial sepsis [222]. While IL-10 repressed the production of NO by macrophages or keratinocytes [223, 224], it did not modify its release by mesangial cells [225] and even enhanced the production of NO by bone marrow derived macrophages and osteoclasts [226, 227]. The inhibitory capacity of IL-10 may also depend on the nature of the triggering agent. For example, the modulatory activity of IL-10 on the proliferation or the cytotoxic activity of $CD8^+$ T lymphocytes was different when cells were activated with allogenic monocytes, anti-CD3 antibodies or IL-2 [228]. IL-10 repressed the LPS-induced IL-8 production by neutrophils but not TNF-α-induced IL-8 production [229]. We have shown in *in vitro* experimental studies that IL-10 primed leukocytes and led to enhanced production of TNF and IL-6 upon further stimulation by LPS. The prevention of monocyte adherence by red cells in the whole-blood assays or by cultures of peripheral blood mononuclear cells on teflon, contributed to this observation [230]. Altogether, these results indicate that IL-10-induced modulation of cytokine production depends on the *in vitro* experimental procedures used and on the *in vivo* localization of the event. A similar effect has been reported concerning the pre-treatment with IL-10 of human cell clones which then produced higher levels of IL-2, IL-4, IFN-γ, and TNF [231]. This observation was shown to be associated with the anti-apoptotic property of IL-10 on T cells. On T lymphocytes infected with human immunodeficiency virus (HIV), IL-10 was shown to act synergistically with TNF to favor viral replication and even to induce the production of TNF [232]. On other cells such as mast cells, IL-10 can behave differently. IL-10 can inhibit cytokine generation by mast cells [233] but can act synergistically with c-Kit ligand to increase cyclooxygenase-2 expression and PGD2 production [234].

IL-6 is also considered as an anti-inflammatory cytokine, mainly for its action on hepatocytes and its capacity to induce the production of acute phase proteins

which are essentially anti-inflammatory. However, in contrast, IL-6 can induce bone resorption [235], muscle atrophy [236], anemia [237] and can prime neutrophils for the production of PAF and superoxide anion [99, 100]. While IL-6 does not activate endothelial cells, it induces MCP-1, MCP-3, and IL-8 production, signal transducer and activator of transcription (STAT)-3 activation, and ICAM-1 expression, in the presence of its soluble receptor which is naturally found in plasma [102].

Another fascinating example of discordance between dogma and reality is given 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 14 to 144 h after the infusion led to far higher levels of TNF and IL-6 as compared with the same volunteers who did not receive the cortisol treatment [238]. Other examples will almost certainly appear in the next few months, illustrating, if necessary, that we still have a lot to understand and should be very careful when analyzing the inflammatory response during SIRS.

Conclusion

The generation of pro-inflammatory cytokines and the capacity of some of them to further induce the production of others within self-amplifying loops plays a major role in the perpetuation of the inflammatory process. However, most inflammatory mediators can be induced by other signals, such as microbial derived products or ischemia, activating the coagulation cascade and the complement system. The capacity of the pro-inflammatory cytokines to further induce a wide type of inflammatory mediator is characteristic of their activities. Of course natural counter-regulation occurs through the generation of acute phase proteins, anti-inflammatory cytokines, endogenous corticoids and heat shock proteins. These later events, together with the reduced capacity of circulating leukocytes to further produce cytokines, may reflect what Louis Pasteur called "Natura medicatrix". When this natural negative regulation appears insufficient, in many animal model of severe inflammation, the therapeutical blocking of the induction or the activities of the major pro-inflammatory cytokines has been demonstrated to be helpful. In human, such approaches have so far been rather disappointing in patients with acute inflammation and systemic inflammatory response syndrome but appear highly promising in some chronic inflammatory diseases such as rheumatoid arthritis or Crohn's disease.

References

1. Echtenacher B, Männel D, Hültner L (1996) Critical protective role of mast cells in a model of acute septic peritonitis. Nature 381:75–77
2. Rush B, Sori A, Murphy T, Smith S, Flanagan J, Machiedo G (1988) Endotoxemia and bacteremia during hemorrhagic shock. The link between trauma and sepsis. Ann Surg 207:549–554
3. Bahrami S, Schlag G, Yao YM, Redl H (1995) Significance of translocation/endotoxin in the development of systemic sepsis following trauma and/or haemorrhage. Prog Clin Biol Res 392:197–208

4. Yao YM, Bahrami S, Leichtfried G, Redl H, Schlag G (1995) Pathogenesis of hemorrhage-induced bacteria/endotoxin translocation in rats. Effects of recombinant bactericidal/permeability-increasing protein. Ann Surg 221:398–405
5. Cabié A, Farkas J-C, Fitting C, et al (1993) High levels of portal TNF-α during abdominal aortic surgery in man. Cytokine 5:448–453
6. Wang W, Smail N, Wang P, Chaudry IH (1998) Increased gut permeability after hemorrhage is associated with upregulation of local and systemic IL-6. J Surg Res 79:39–46
7. Le Tulzo Y, Shenkar R, Kaneko D, et al (1997) Hemorrhage increase cytokine expression in lung mononuclear cells in mice. J Clin Invest 99:1516–1524
8. Zhu XL, Ayala A, Zellweger R, Morrison MH, Chaudry IH (1994) Peritoneal macrophages show increased cytokine gene expression following haemorrhagic shock. Immunology 83: 378–383
9. Frangogiannis NG, Lindsey ML, Michael LH, et al (1998) Resident cardiac mast cells degranulate and release preformed TNF, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 98:699–710
10. Suzuki S, Toledo-Peryera LH (1994) Interleukin-1 and tumor necrosis factor production as the initial stimulants of liver ischemia and reperfusion. J Surg Res 57:253–258
11. Safirstein R, Megyesi J, Saggi SJ, et al (1991) Expression of cytokine-like genes JE and KC is increased during renal ischemia. Am J Physiol 261:F1095–F1101
12. Ghezzi P, Dinarello CA, Bianchi M, Rosandich ME, Repine JE, White CW (1991) Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 3:189–194
13. Shreeniwas R, Koga S, Karakurum M, et al (1992) Hypoxia-mediated induction of endothelial cell interleukin-1α. J Clin Invest 90:2333–2339
14. Karakurum M, Shreeniwas R, Chen J, et al (1994) Hypoxic induction of interleukin-8 gene expression in human endothelial cells. J Clin Invest 93:1564–1570
15. Haeffner-Cavaillo N, Rousselier N, Pnozio O, et al (1989) Induction of IL-1 production in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 98:1100–1106
16. Burns S, Newburger J, Xiao M, Mayer J, Walsh A, Neufeld E (1995) Induction of interleukin-8 messenger RNA in heart and skeletal muscle during pediatric cardiopulmonary bypass. Circulation 92 :II-315–II-321
17. Wan S, Desmet JM, Barvais L, Goldstein M, Vincent JL, Leclerc JL (1996) Myocardium is a major source of proinflammatory cytokines in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 112:806–811
18. Gross V, Andreesen R, Leser H-G, et al (1992) Interleukin-8 and neutrophil activation in acute pancreatitis. Eur J Clin Invest 22:200–203
19. Giulian D, Lachman LB (1985) Interleukin-1 stimulation of astroglial proliferation after brain injury. Science 228:497–499
20. Holmin S, Schalling M, Höjeberg B, Nordqvist AC, Skeftruna AK, Mathiesen T (1997) Delayed cytokine expression in rat brain following experimental contusion. J Neurosurg 86: 493–504
21. Keel M, Ecknauer E, Stocker R, et al (1996) Different pattern of local and systemic release of proinflammatory and anti-inflammatory mediators in severely injured patients with chest trauma. J Trauma 40:907–914
22. Badia JM, Whawell SA, Scott-Coombes DM, Abel PD, Williamson RCN, Thompson JN (1996) Peritoneal and systemic cytokine response to laparotomy. Br J Surg 83:347–348
23. Dehoux MS, Boutten A, Ostinelli J, et al (1994) Compartmentalized cytokine production within the human lung in unilateral pneumonia. Am J Respir Crit Care Med 150:710–716
24. Kyan-Aung U, Haskard DO, Poston RN, Thornhill MH, Lee TH (1991) ELAM-1 and ICAM-1 mediate the adhesion of eosinophils to endothelial cells in vitro and are expressed by endothelium in allergic cutaneous inflammation in vivo. J Immunol 146:521–528
25. Mulligan MS, Jones ML, Bolanowski MA, et al (1993) Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody. J Immunol 150:5585–5595
26. Huffnagle GB, Strieter RM, Standiford TJ, et al (1995) The role of monocyte chemotactic protein-1 in the recruitment of monocytes and CD4+ T cells during a pulmonary *Cryptococcus neoformans* infection. J Immunol 155:4790–4797

27. van der Poll T, Buller HR, ten Cate H, et al (1990) Activation of coagulation after administration of tumor necrosis factor to normal subjects. N Engl J Med 322:1622–1627
28. Okusawa S, Gelfand J, Lkejima T, Connolly R, Dinarello C (1988) Interleukin 1 induces a shock like state in rabbit. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 81:1162–1172
29. Goldblum SE, Yoneda K, Cohen DA, McClain CJ (1988) Provocation of pulmonary vascular endothelial injury in rabbits by human recombinant IL-1 beta. Infect Immun 56:2255–2263
30. Fischer E, Marano MA, Barber AE, et al (1991) Comparison between effects of interleukin-1 administration and sublethal endotoxemia in primates. Am J Physiol 261:R442–R452
31. Ohlsson K, Björk P, Bergenfield M, Hageman R, Thompson R (1990) Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 348:550–552
32. Alexander HR, Doherty GM, Buresh CM, Venzon DJ, Norton JA (1991) A recombinant human receptor antagonist to interleukin-1 improves survival after lethal endotoxemia in mice. J Exp Med 173:1029–1032
33. Aiura K, Gelgand J, Burke J, Thompson R, Dinarello C (1993) Interleukin-1 receptor antagonist prevents S. epidermidis hypotension and reduces circulating levels of TNF and IL-1β in rabbits. Infect Immun 61:3342–3350
34. Fischer E, Marano MA, Van Zee KJ, et al (1992) Interleukin-1 receptor blockade improves survival and hemodynamic performance in Escherichia coli septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 89:1551–1557
35. Mancilla J, Garcia P, Dinarello CA (1993) The IL-1 receptor antagonist can either reduce or enhance the lethality of *Klebsiella pneumoniae* sepsis in newborn rats. Infect Immun 61: 926–932
36. Hirsch E, Irikura VM, Paul SM, Hirsh D (1996) Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. Proc Natl Acad Sci USA 93:11 008–11 013
37. Li P, Allen H, Banerjee S, et al (1995) Mice deficient in IL-1β converting enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. Cell 80:401–411
38. Fantuzzi G, Ku G, Harding MW, et al (1997) Response to local inflammation of IL-1-beta-converting enzyme-deficient mice. J Immunol 158:1818–1824
39. Fantuzzi G, Hui Z, Faggioni R, et al (1996) Effect of endotoxin in IL-1β deficient mice. J Immunol 157:291–296
40. Natanson C, Eichenholz PW, Danner RL, et al (1989) Endotoxin and TNF challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med 169:823–832
41. Van der Poll T, Romijn JA, Endert E, Borm JJ, Buller HR, Sauerwein HP (1991) TNF mimics the metabolic response to acute infection in healthy humans. Am J Physiol 261:E457–E465
42. Ciancio MJ, Hunt J, Jones SB, Filkins JP (1991) Comparative and interactive in vivo effects of tumor necrosis factor alpha and endotoxin. Circ Shock 33:108–120
43. Eichacker PQ, Hoffman WD, Farese A, et al (1991) TNF but not IL-1 in dogs causes lethal lung injury and multiple organ dysfunction similar to human sepsis. J Appl Physiol 71:1979–1989
44. Edwards MJ, Heniford BT, Miller FN (1993) Tumor necrosis factor mediates disseminated intravascular inflammation in the genesis of multiple organ edema. J Surg Res 54:140–144
45. Oldhafer KJ, Kuse ER (1998) High dose tumor necrosis factor alpha leads to the systemic inflammatory response syndrome. Am J Med 105:346–347
46. Schilling PJ, Murray JL, Markowitz AB (1992) Novel tumor necrosis factor toxic effects. Pulmonary hemorrhage and severe hepatic dysfunction. Cancer 69:256–260
47. Doherty GM, Lange JR, Langstein HN, Alexander HR, Buresh CM, Norton JA (1992) Evidence for IFN-gamma as a mediator of the lethality of endotoxin and tumor necrosis factor-alpha. J Immunol 149:1666–1670
48. Rothstein JL, Schreiber H (1988) Synergy between TNF and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. Proc Natl Acad Sci USA 85:607–611
49. Hrushesky WJM, Langevin T, Kim YJ, Wood PA (1994) Circadian dynamics of tumor necrosis factor alpha lethality. J Exp Med 180:1059–1065
50. Kahky MP, Daniel CO, Cruz AB, Gaskill HV (1990) Portal infusion of tumor necrosis factor increases mortality in rats. J Surg Res 49:138–145
51. Beutler B, Milsark IW, Cerami AC (1985) Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 229:869–871

52. Tracey KJ, Fong Y, Hesse DG, et al (1987) Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330:662–664
53. Caty MG, Guice KS, Oldham KT, Remick DG, Kunkel SI (1990) Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. Ann Surg 212:694–700
54. Van Zee K, Kohno T, Fisher E, Rock C, Moldawer L, Lowry S (1992) TNF soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor α *in vitro* and *in vivo*. Proc Natl Acad Sci USA 89:4845–4849
55. Lesslauer W, Tabuchi H, Gentz R, et al (1991) Recombinant soluble tumor necrosis factor receptor proteins protect mice from LPS-induced lethality. Eur J Immunol 21:2883–2886
56. Ashkenazi A, Marsters S, Capon D, et al (1991) Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin. Proc Natl Acad Sci USA 88:10535–10539
57. Pfeffer K, Matsuyama T, Kundig TM, et al (1993) Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell 73:457–467
58. Erickson SL, de Sauvage F, Kikly K, et al (1994) Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. Nature 372: 560–563
59. Amiot F, Fitting C, Tracey KJ, Cavaillon J-M, Dautry F (1997) LPS-induced cytokine cascade and lethality in LTα/TNF-α deficient mice. Mol Med 3:864–875
60. Bryant D, Becker L, Richardson J, et al (1998) Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor. Circulation 97:1375–1381
61. Probert L, Akassoglou K, Kassiotis G, Pasparakis M, Alexopoulou L, Kollias G (1997) TNF-alpha transgenic and knockout models of CNS inflammation and degeneration. J Neuroimmunol 72:137–141
62. Butler DM, Malfait AM, Mason LJ, et al (1997) DBA/1 mice expressing the human TNF transgen develop a severe erosive arthritis. J Immunol 159:2867–2876
63. Ryffel B (1997) Interleukin-12: role of interferon-γ in IL-12 adverses effects. Clin Immunol Immunopath 83:18–20
64. Wysocka M, Kubin M, Vieira LQ, et al (1995) IL-12 is required for interferon-gamma production and lethality in lipopolysaccharide-induced shock in mice. Eur J Immunol 25: 672–676
65. Heinzel FP (1990) The role of interferon-gamma in the pathology of experimental endotoxemia. J Immunol 145:2920–2924
66. Silva AT, Cohen J (1992) Role of interferon-gamma in experimental Gram-negative sepsis. J Infect Dis 166:331–335
67. Kamijo R, Le J, Shapiro D, et al (1993) Mice that lack the interferon-gamma receptor have profoundly altered responses to infection with Bacillus Calmette-Guerin and subsequent challenge with lipopolysaccharide. J Exp Med 178:1435–1440
68. Car BD, Eng VM, Schnyder B, et al (1994) Interferon-gamma receptor deficient mice are resistant to endotoxic shock. J Exp Med 179:1437–1444
69. Waring P, Wycherley K, Cary D, Nicola N, Metcalf D (1992) Leukemia inhibitory factor levels are elevated in septic shock and various inflammatory body fluids. J Clin Invest 90: 2031–2037
70. Block MI, Berg M, McNamara MJ, Norton JA, Fraker DL, Alexander HR (1993) Passive immunization of mice against D factor blocks lethality and cytokine release during endotoxemia. J Exp Med 178:1085–1090
71. Guillet C, Fourcin M, Chevalier S, Pouplard A, Gascan H (1995) ELISA detection of circulating levels of LIF, OSM and CNTF in septic shock. Ann NY Acad Sci 762:407–412
72. Modur V, Feldhaus MJ, Weyrich AS, et al (1997) Oncostanin M is a proinflammatory mediator. In vivo effects correlates with endothelial cell expression of inflammatory cytokines and adhesion molecules. J Clin Invest 100:158–168
73. Bernhagen J, Calandra T, Mitchell RA, et al (1993) MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature 365:756–759
74. Bacher M, Meinhardt A, Lan HY, et al (1997) Migration inhibitory factor expression in experimentally induced endotoxemia. Am J Pathol 150:235–246

75. Sutton ET, Norman JG, Newton CA, Hellermann GR, Richards IS (1997) Endothelial structural integrity is maintained during endotoxic shock in an interleukin-1 type 1 receptor knockout mouse. Shock 7:105–110
76. Tedder TF, Steeber DA, Pizcueta P (1995) L-selectin-deficient mice have impaired leukocyte recruitment into inflammatory sites. J Exp Med 181:2259–2264
77. Xu H, Gonzalo J, St Pierre Y, et al (1994) Leucocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. J Exp Med 180:95–109
78. Broaddus VC, Boylan AM, Hoeffel JM, Kim KJ, Sadick M, Chuntharapai A (1994) Neutralization of IL-8 inhibits neutrophils influx in a rabbit model of endotoxin-induced pleurisy. J Immunol 152:2960–2967
79. Marty C, Misset B, Tamion F, Fitting C, Carlet J, Cavaillon J-M (1994) Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. Crit Care Med 22:673–679
80. Marie C, Fitting C, Cheval C, et al (1997) Presence of high levels of leukocyte-associated IL-8 upon cell activation and in patients with sepsis syndrome. Infect Immun 65:865–871
81. Gimbrone MA, Obin MS, Brock AF, et al (1989) Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. Science 246:1601–1603
82. Solomkin JS, Bass RC, Bjornson HS, Tindal CJ, Babcock GF (1994) Alterations of neutrophil responses to TNF-α and IL-8 following human endotoxemia. Infect Immun 62:943–947
83. Cunha FQ, Cunha Tamashiro WMS (1992) Tumour necrosis factor-alpha and interleukin-8 inhibit neutrophil migration in vitro and in vivo. Mediators Inflamm 1:397–401
84. Zisman DA, Kunkel SL, Strieter RM, et al (1997) MCP-1 protects mice in lethal endotoxemia. J Clin Invest 99:2832–2836
85. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T (1989) The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin-6, interleukin-1, and fatal outcome. J Exp Med 169:333–338
86. Hack C, de Groot E, Felt-Bersma R, et al (1989) Increased plasma levels of interleukin-6 in sepsis. Blood 74:1704–1710
87. Muñoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon J-M (1991) Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 21:2177–2184
88. Calandra T, Gerain J, Heumann D, Baumgartner JD, Glauser MP (1991) High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. Am J Med 91:23–29
89. Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW (1994) Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood 83:113–118
90. Gabay C, Smith MF, Eidlen D, Arend WP (1997) Interleukin-1 receptor antagonist is an acute phase protein. J Clin Invest 99:2930–2940
91. Tilg H, Vannier E, Vachino G, Dinarello CA, Mier JW (1993) Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells. J Exp Med 178:1629–1636
92. Heuertz RM, Piquette CA, Webster RO (1993) Rabbits with elevated serum C-reactive protein exhibit diminished neutrophil infiltration and vascular permeability in C5a-induced alveolitis. Am J Pathol 142:319–328
93. Moore DF, Rosenfeld MR, Gribbon PM, Winlove CP, Tsai CM (1997) Alpha-1 acid glycoprotein (orosomucoid): interaction with bacterial lipopolysaccharide and protection from sepsis. Inflammation 21:69–82
94. Libert C, Vanmolle W, Brouckaert P, Fiers W (1996) Alpha1-antitrypsin inhibits the lethal response to TNF in mice. J Immunol 157:5126–5129
95. Libert C, Brouckaert P, Fiers W (1994) Protection by alpha 1-acid glycoprotein against tumor necrosis factor-induced lethality. J Exp Med 180:1571–1575
96. Mancuso G, Tomasello F, Migliardo M, et al (1994) Beneficial effects of interleukin-6 in neonatal mouse models of group B streptococcal disease. Infect Immun 62:4997–5002
97. Barton BE, Jackson JV (1993) Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. Infect Immun 61:1496–1499

98. Yoshizawa KI, Naruto M, Ida N (1996) Injection time of interleukin-6 determines fatal outcome in experimental endotoxin shock. J Interferon Cytokine Res 16:995–1000
99. Borish L, Rosenbaum R, Albury L, Clark S (1989) Activation of neutrophils by recombinant interleukin-6. Cell Immunol 121:280–289
100. Biffl WL, Moore EE, Moore FA, Barnett CC, Silliman CC, Peterson VM (1996) Interleukin-6 stimulates neutrophil production of platelet activating factor. J Leuk Biol 59:569–574
101. Denis M (1992) Interleukin-6 in mouse hypersensitivity pneumonitis: changes in lung free cells following depletion of endogenous IL-6 or direct administration of IL-6. J Leuk Biol 52:197–201
102. Romano M, Sironi M, Toniatti C, et al (1997) Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 6:315–325
103. Gabay C, Singwe M, Genin B, et al (1996) Circulating levels of IL-11 and leukaemia inhibitory factor (LIF) do not significantly participate in the production of acute-phase proteins by the liver. Clin Exp Immunol 105:260–265
104. Du XX, Doerschuk CM, Orazi A, Williams DA (1994) A bone marrow stromal-derived growth factor, interleukin-11, stimulates recovery of small intestinal mucosal cells after cytoablative therapy. Blood 83:33–37
105. Chang M, Williams A, Ishizawa L, Knoppel A, van de Ven C, Cairo MS (1996) Endogenous interleukin-11 (IL-11) expression is increased and prophylactic use of exogenous IL-11 enhances platelet recovery and improves survival during thrombocytopenia associated with experimental group B streptococcal sepsis in neonatal rats. Blood Cells Mol Dis 22:57–67
106. Khabar KSA, ElBarbary MA, Khouqeer F, Devol E, Al-Gain S, Al-Halees Z (1997) Circulating endotoxin and cytokines after cardiopulmonary bypass: differential correlation with duration of bypass and systemic response/multiple organ dysfunction syndromes. Clin Immunol Immunopathol 85:97–103
107. Grewal HP, Mohey el Din A, Gaber L, Kotb M, Gaber AO (1994) Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF polyclonal antibody. Am J Surg 167:214–219
108. Hughes CB, Grewal HP, Gaber LW, et al (1996) Anti-TNF therapy improves survival and ameliorates the pathophysiological sequelae in acute pancreatitis in the rat. Am J Surg 171: 274–280
109. Squadrito F, Altavilla D, Ioculano M, et al (1992) Passive immunization with antibodies against tumor necrosis factor protects from the lethality of splanchnic occlusion shock. Circ Shock 37:236–244
110. Imagawa DK, Millis JM, Seu P, et al (1991) The role of tumor necrosis factor in allograft rejection. III. Evidence that anti-TNF antibody therapy prolongs allograft survival in rats with acute rejection. Transplantation 51:57–62
111. Fraker DJ, Langstein HN, Norton JA (1989) Passive immunization against tumor necrosis factor partially abrogates interleukin-2 toxicity. J Exp Med 170:1015–1020
112. Welbourn R, Goldman G, O'Riordain M, et al (1991) Role of tumor necrosis factor as mediator of lung injury following lower torso ischemia. J Appl Physiol 70:2645–2649
113. Collettti LM, Remick DG, Burtch GD, Kunkel SL, Strieter RM, Campbell DA (1990) Role of tumor necrosis factor in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 85:1936–1943
114. Zingarelli B, Squadrito F, Altavilla D, Calapai G, Di Rosa M, Caputi AP (1994) Role of TNF in acute hypovolemic hemorrhagic shock in rats. Am J Physiol 266:H1512–H1515
115. Bahrami S, Yao YM, Leichtfried G, Redl H, Marzi I, Schlag G (1997) Significance of TNF in hemorrhage-related hemodynamic alteration, organ injury and mortality in rats. Am J Physiol 272:H2219–H2226
116. Jansen MJJM, Hendriks H, Hermsen R, van der Meer JWM, Goris RJA (1998) A monoclonal antibody against tumour necrosis factor improves survival in experimental multiple organ dysfunction syndrome. Cytokine 10:904–910
117. Elliot MJ, Maini RN, Feldmann M, et al (1994) Randomised double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor versus placebo in rheumatoid arthritis. Lancet 344:1105–1110

118. van Dullemen HM, van Deventer SJ, Hommes DW, et al (1995) Treatment of Crohn's disease with anti-TNF chimeric monoclonal antibody (cA2). Gastroenterology 109:129–135
119. Fong Y, Tracey KJ, Moldawer LL, et al (1989) Antibodies to cachectin/tumor necrosis factor reduce IL-1β and IL-6 appearance during lethal bacteremia. J Exp Med 170:1627–1633
120. Norman JG, Franz MG, Fink GS, et al (1995) Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 221:625–634
121. Pugin J, Ricou B, Steinberg KP, Suter PM, Martin TR (1996) Proinflammatory activity in bronchoalveolar lavage fluids from patients with ARDS, a prominent role for interleukin-1. Am J Respir Crit Care Med 153:1850–1856
122. Cominelli F, Nast CC, Clark BD, et al (1990) IL-1 gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. J Clin Invest 86:972–980
123. Shanley TP, Peters JL, Jones ML, Chensue SW, Kunkel SL, Ward PA (1996) Regulatory effects of endogenous interleukin-1 receptor antagonist protein in immunoglobulin G immune complex-induced lung injury. J Clin Invest 97:963–970
124. Abraham E, Allbee J (1994) Effects of therapy with IL-1ra on pulmonary cytokine expression following hemorrhage and resuscitation. Lymphokine Cytokine Res 13:343–347
125. Marsh CB, Moore SA, Pope MHA, Wewers MD (1994) IL-1ra supresses endotoxin-induced IL-1 and TNF release from mononuclear phagocytes. Am J Physiol 267:L39–L45
126. Wulczyn FG, Krappmann D, Scheidereit C (1996) The NF-κB/Rel and IκB families: mediators of immune response and inflammation. J Mol Med 74:749–769
127. Baldwin AS, Jr (1996) The NF-κB and IκB proteins: new discoveries and insights. Ann Rev Immunol 14:649–683
128. Ghosh S, May MJ, Kopp EB (1998) NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Ann Rev Immunol 16:225–260
129. Schmitz ML, Baeuerle PA (1991) The p65 subunit is responsible for the strong transcription activating potential of NF-κB. EMBO J 10:3805–3817
130. Malinin NL, Boldin MP, Kovalenko AV, Wallach D (1997) MAP3K-related kinase involved in NF-κB induction by TNF, CD95 and IL-1. Nature 385:540–544
131. Hsu H, Xiong J, Goeddel DV (1995) The TNF receptor 1–associated protein TRADD signals cell death and NF-activation. Cell 81:495–504
132. Rothe M, Wong SC, Henzel WJ, Goeddel DV (1994) A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 KDa tumor necrosis factor receptor. Cell 78:681–692
133. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV (1996) TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-signaling complex. Immunity 4:387–396
134. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G (1995) Molecular cloning and characterization of a second subunit of the interleukin-1 receptor complex. J Biol Chem 270:13757–13765
135. Burns K, Martinon F, Esslinger C, et al (1998) MyD88, an adapter protein involved in interleukin-1 signaling. J Biol Chem 273:12203–12209
136. Lee FS, Hagler J, Chen J, Maniatis T (1997) Activation of the IκBα kinase complex by MEKK1, a kinase of the JNK pathway. Cell 88:213–222
137. Hirano M, Osada S-I, Aoki T, et al (1996) MEK kinase is involved in tumor necrosis factor α-induced NF-κB activation and degradation of IκB-α. J Biol Chem 271:13234–13238
138. Liu Z, Hsu H, Goeddel DV, Karin M (1996) Dissection of the TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. Cell 87:565–576
139. Di Donato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M (1997) A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. Nature 388:548–554
140. Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M (1997) The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. Cell 91:243–252
141. Rothwarf DM, Zandi E, Natoli G, Karin M (1998) IKK-γ is an essential regulatory subunit of the IκB kinase complex. Nature 395:297–300
142. Westwick JK, Weitzel C, Minden A, Karin M, Brenner DA (1994) TNF-α stimulates AP-1 activity through prolonged activation of the c-jun kinase. J Biol Chem 269:26396–26401

143. Raingeaud J, Gupta S, Rogers JS, et al (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 270:7420–7426
144. Wesselborg S, Bauer MKA, Vogt M, Schmitz ML, Schulze-Osthoff K (1997) Activation of transcription factor NF-κB and p38 mitogen-activated protein kinase is mediated by distinct and separate stress effector pathways. J Biol Chem 272:12422–12429
145. Yuasa T, Ohno S, Kehrl JH, Kyriakis JM (1998) TNF signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. J Biol Chem 273:22681–22692
146. Kanakaraj P, Schafer PH, Cavender DE, et al (1998) IL-1 receptor-associated kinase (IRAK) requirement for optimal induction of multiple IL-1 signaling pathways and IL-6 production. J Exp Med 187:2073–2079
147. Marchant A, Devière J, Byl B, De Groote D, Vincent J, Goldman M (1994) Interleukin-10 production during septicaemia. Lancet 343:707–708
148. Marie C, Cavaillon J-M, Losser M-R (1996) Elevated levels of circulating transforming growth factor-β1 in patients with the sepsis syndrome. Ann Intern Med 125:520–521
149. Ayala A, Lehman DL, Herdon CD, Chaudry IH (1994) Mechanism of enhanced susceptibility to sepsis following hemorrhage. Interleukin-10 suppression of T-cell response is mediated by eicosanoid-induced interleukin-4 release. Arch Surg 129:1172–1178
150. Brandtzaeg P, Osnes L, Øvstebø R, Joø GB, Westwik AB, Kierulf P (1996) Net inflammatory capacity of human septic shock plasma evaluated by a monocyte-based target cell assay: identification of IL-10 as a major functional deactivator of human monocytes. J Exp Med 184:51–60
151. Miller-Graziano CL, Szabo G, Griffey K, Mehta B, Kodys K, Catalano D (1991) Role of elevated monocyte TGFβ production in post-trauma immunosuppression. J Clin Immunol 11:95–102
152. Ayala A, Meldrum DR, Perrin MM, Chaudry IH (1993) The release of transforming growth factor-beta following haemorrhage: its role as a mediator of host immunosuppression. Immunology 79:479–484
153. Ayala A, Knotts JB, Ertel W, Perrin MM, Morrison MH, Chaudry IH (1993) Role of interleukin 6 and transforming growth factor-beta in the induction of depressed splenocyte responses following sepsis. Arch Surg 128:89–94
154. Sheeran P, Hall GM (1997) Cytokines in anaesthesia. Br J Anaesth 78:201–219
155. Bencsics A, Elenkov IJ, Vizi ES (1997) Effect of morphine on lipopolysaccharide-induced tumor necrosis factor-alpha production in vivo: Involvement of the sympathetic nervous system. J Neuroimmunol 73:1–6
156. Hill GE, Pohorecki R, Alonso A, Rennard SI, Robbins RA (1996) Aprotinin reduces interleukin-8 production and lung neutrophil accumulation after cardiopulmonary bypass. Anesth Analg 83:696–700
157. McCall CE, Grosso-Wilmoth LM, LaRue K, Guzman RN, Cousart SL (1993) Tolerance to endotoxin-induced expression of the interleukin-1β gene in blood neutrophils of humans with the sepsis syndrome. J Clin Invest 91:853–861
158. Cavaillon JM (1995) The nonspecific nature of endotoxin tolerance. Trends Microbiol 3:320–324
159. Helminen M (1991) Interleukin-1 production from peripheral blood monocytes in septic infections in children. Scand J Infect Dis 23:607–611
160. Muñoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM (1991) Dysregulation of in vitro cytokine production by monocytes during sepsis. J Clin Invest 88:1747–1754
161. Sekatrian JC, Yee J, Christou NV (1994) Reduced tumor necrosis factor-α production in lipopolysaccharide-treated whole blood from patients in the intensive care unit. Arch Surg 129:187–192
162. Van Deuren M, Van Der Ven-Jongekrijg H, Demacker PNM, et al (1994) Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. J Infect Dis 169:157–161
163. Marchant A, Alegre M, Hakim A, et al (1995) Clinical and biological significance of interleukin-10 plasma levels in patients with septic shock. J Clin Immunol 15:265–272
164. Randow F, Syrbe U, Meisel C, et al (1995) Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor β. J Exp Med 181:1887–1892

165. Ertel W, Keel M, Neidhardt R, et al (1997) Inhibition of the defense system stimulating interleukin-12 interferon-γ pathway during critical illness. Blood 89:1612–1620
166. Luger A, Graf H, Schwarz HP, Stummvoll HK, Luger TA (1986) Decreased serum IL-1 activity and monocyte IL-1 production in patients with fatal sepsis. Crit Care Med 14:458–461
167. Cavaillon J-M, Muñoz C, Marty C, et al (1993) Cytokine production by monocytes from patients with sepsis syndrome and by endotoxin-tolerant monocytes. In: Levin J, Alving CR, Munford RS, Stütz PL (eds) Bacterial endotoxin: recognition and effector mechanisms. Elsevier Sc. Publ., New York, pp 275–284
168. Astiz M, Saha D, Lustbader D, Lin R, Rackow E (1996) Monocyte response to bacterial toxins, expression of cell surface receptors, and release of anti-inflammatory cytokines during sepsis. J Lab Clin Med 128:597–600
169. Cabié A, Fitting C, Farkas J-C, et al (1992) Influence of surgery on in-vitro cytokine production by human monocytes. Cytokine 4:576–580
170. Kleinschmidt S, Wanner GA, Bußmann D, et al (1998) Proinflammatory cytokine gene expression in whole blood from patients undergoing coronary artery bypass surgery and its modulation by pentoxifylline. Shock 9:12–20
171. Faist E, Mewes A, Strasser T, et al (1988) Alteration of monocyte function following major injury. Arch Surg 123:287–292
172. Marie C, Muret J, Fitting C, Losser M-R, Payen D, Cavaillon J-M (1998) Reduced ex vivo interleukin-8 production by neutrophils in septic and non-septic systemic inflammatory response syndrome. Blood 91:3439–3446
173. Wood J, Rodrick M, O'Mahony J, et al (1984) Inadequate interleukin 2 production. A fundamental immunological deficiency in patients with major burns. Ann Surg 200:311–320
174. Rodrick MM, Wood JJ, O'Mahony JB, et al (1986) Mechanisms of immunosupression associated with severe nonthermal traumatic injuries in man: production of interleukin-1 and 2. J Clin Immunol 6:310–318
175. Grzelak I, Olszewski WL, Rowinski W (1989) Blood mononuclear cell production of IL-1 and IL-2 following moderate surgical trauma. Eur Surg Res 21:114–122
176. Hisatomi K, Isomura T, Kawara T, et al (1989) Changes in lymphocyte subsets, mitogen responsiveness, and interleukin-2 production after cardiac operations. J Thorac Cardiovasc Surg 98:580–591
177. Abraham E, Chang YH (1992) Haemorrhage-induced alteration in function and cytokine production of T cells and T cell subpopulations. Clin Exp Immunol 90:497–502
178. Hershman MJ, Cheadle WG, Wellhausen SR, Davidon P, Polk HC (1990) Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. Br J Surg 77: 204–207
179. Ertel W, Keel M, Steckholzer U, Ungethüm U, Trentz O (1996) IL-10 attenuates the release of pro-inflammatory cytokines but depresses splenocyte function in murine endotoxemia. Arch Surg 131:51–56
180. Meldrum DR, Ayala A, Perrin MM, Ertel W, Chaudry IH (1991) Diltiazem restores IL-2, IL-3, IL-6 and IFN-gamma synthesis and decreases host suceptibility to sepsis following hemorrhage. J Surg Res 51:158–164
181. Ayala A, Deol ZK, Lehman DL, Herdon CD, Chaudry IH (1994) Polymicrobial sepsis but not low-dose endotoxin infusion causes decreased splenocyte IL-2/IFN-gamma release while increasing IL-4/IL-10 production. J Surg Res 56:579–585
182. Mack VE, McCarter MD, Naama HA, Calvano SE, Daly JM (1996) Dominance of T-helper 2-type cytokine after severe injury. Arch Surg 131:1303–1309
183. O'Sullivan ST, Lederer JA, Horgan AF, Chin DHL, Mannick JA, Rodrick ML (1995) Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222: 482–492
184. Naldini A, Borrelli E, Cesari S, Giomarelli P, Toscano M (1995) In vitro cytokine production and T-cell proliferation in patients undergoing cardiopulmonary by-pass. Cytokine 7: 165–170
185. Miller-Graziano CL, De AK, Kodys K (1995) Altered IL-10 levels in trauma patients monocytes and T lymphocytes. J Clin Immunol 15:93–104

186. Napolitano L, Campbell C (1995) Polymicrobial sepsis following trauma inhibits interleukin-10 secretion and lymphocyte proliferation. J Trauma 39:104–111
187. Ertel W, Morrison MH, Ayala A, Dean R, Chaudry IH (1992) Interferon-γ attenuates hemorrhage-induced suppression of macrophage and splenocyte functions and decreases susceptibility to sepsis. Surgery 111:177–187
188. Dries DJ, Jurkovich GJ, Maier RV, et al (1994) Effect of interferon-gamma on infection-related death in patients with severe injuries. A randomized, double-blind, placebo-controlled trial. Arch Surg 129:1031–1041
189. Döcke WD, Randow F, Syrbe U, et al (1997) Monocyte deactivation in septic patients: restoration by IFNγ treatment. Nature Med 3:678–681
190. Mendez M, Molloy R, O'Rioirdain D, et al (1993) Lymphokine activated killer cells enhance IL-2 prevention of sepsis-related death in a murine model of thermal injury. J Surg Res 54: 565–570
191. Bundschuh DS, Barsig J, Hartung T, et al (1997) Granulocyte-macrophage colony-stimulating factor and IFN-gamma restore the systemic TNF-alpha response to endotoxin in lipopolysaccharide-desensitized mice. J Immunol 158:2862–2871
192. Randow F, Docke WD, Bundschuh DS, Hartung T, Wendel A, Volk HD (1997) In vitro prevention and reversal of lipopolysaccharide desensitization by IFN-gamma, IL-12, and granulocyte-macrophage colony-stimulating factor. J Immunol 158:2911–2918
193. Inoue T, Saito H, Fukushima R, et al (1995) Growth hormone and insulin like growth factor I enhance host defense in a murine sepsis model. Arch Surg 130:1115–1122
194. Zellweger R, Zhu XH, Wichmann MW, Ayala A, DeMaso CM, Chaudry IH (1996) Prolactin administration following hemorrhagic shock improves macrophage cytokine release capacity and decreases mortality from subsequent sepsis. J Immunol 157:5748–5754
195. Pociot F, Briant L, Jongeneel CV, et al (1993) Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNFα and TNFβ by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 23:224–231
196. Danis VA, Millington M, Hyland VJ, Grennan D (1995) Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 99:303–310
197. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV (1997) An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 24: 1–8
198. Walley AJ, Cookson W (1996) Investigation of an interleukin-4 promoter polymorphism for associations with asthma and atopy. J Med Genet 33:689–692
199. Bender JR, Sadeghi MM, Watson C, Pfau S, Pardi R (1994) Heterogeneous activation thresholds to cytokines in genetically distinct endothelial cells: evidence for diverse transcriptional responses. Proc Natl Acad Sci USA 91:3994–3998
200. Liu J, Marino MW, Wong G, et al (1998) TNF is a potent anti-inflammatory cytokine in auto-immune-mediated demyelination. Nature Med 4:78–83
201. Erwig L-P, Kluth DC, Walsh GM, Rees AJ (1998) Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines. J Immunol 161: 1983–1988
202. Hodge-Dufour J, Marino MW, Horton ME, et al (1998) Inhibition of interferon-γ induced interleukin-12 production: a potential mechanism for the anti-inflammatory activities of tumor necrosis factor. Proc Natl Acad Sci USA 95:13806–13811
203. Kasama T, Yamazaki J, Hanaoka R, et al (1999) Biphasic regulation of the development of murine type II collagen-induced arthritis by interleukin-12: Possible involvement of endogenous interleukin-10 and tumor necrosis factor alpha. Arthritis Rheum 42:100–109
204. Sherry B, Espinoza M, Manogue KR, Cerami A (1998) Induction of the chemokine β peptides, MIP-1α and MIP-1β by lipopolysaccharide is differentially regulated by immunomodulatory cytokines γ-IFN, IL-10, IL-4 and TGFβ. Mol Med 4:654–657
205. Schleimer RP, Sterbinsky SA, Kaiser J, et al (1992) IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. J Immunol 148:1086–1092

206. De Beaux AC, Maingay JP, Ross JA, Fearon KC, Carter DC (1995) Interleukin-4 and interleukin-10 increase endotoxin-stimulated human umbilical vein endothelial cell interleukin-8 release. J Interferon Cytokine Res 15:441–445
207. Goebeler M, Schnarr B, Toksoy A, et al (1997) Interleukin-13 selectively induces monocyte chemoattractant protein-1 synthesis and secretion by human endothelial cells. Involvement of IL-1Rα and Stat-6 phosphorylation. Immunology 91:450–457
208. Kambayashi T, Jacob CO, Strassmann G (1996) IL-4 and IL-13 modulate IL-10 release in endotoxin-stimulated murine peritoneal mononuclear phagocytes. Cell Immunol 171:153–158
209. D'Andrea A, Ma X, Aste-Amezaga M, Paganin C, Trinchieri G (1995) Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor alpha production. J Exp Med 181:537–546
210. Minty A, Ferrara P, Caput D (1997) Interleukin-13 effects on activated monocytes lead to novel cytokine secretion profiles intermediate between those induced by interleukin-10 and by γ-interferon. Eur Cytokine Netw 8:189–201
211. Hart PH, Jones CA, Finlay-Jones JJ (1995) Monocytes cultured in cytokine-defined environments differ from freshly isolated monocytes in their responses to IL-4 and IL-10. J Leukoc Biol 57:909–918
212. Marfaing-Koka A, Maravic M, Humbert M, Galanaud P, Emilie D (1996) Contrasting effects of IL-4, IL-10 and corticosteroids on RANTES production by human monocytes. Int Immunol 8:1587–1594
213. Marie C, Fitting C, Muret J, Payen D, Cavaillon J-M (1999) Interleukin-8 production in whole blood assays: is interleukin-10 responsible for the downregulation observed in sepsis? Cytokine (in press)
214. Kumar NM, Rabadi NH, Sigurdson LS, Schunemann HJ, Lwebuga-Mukasa JS (1996) Induction of interleukin-1 and interleukin-8 mRNAs and proteins by TGF beta(1) in rat lung alveolar epithelial cells. J Cell Physiol 169:186–199
215. Qian SG, Li W, Li YP, et al (1996) Systemic administration of cellular interleukin-10 can exacerbate cardiac allograft rejection in mice. Transplantation 62:1709–1714
216. Rosenbaum JT, Angell E (1995) Paradoxical effects of IL-10 in endotoxin-induced uveitis. J Immunol 155:4090–4094
217. Moritani M, Yoshimoto K, Tashiro F, et al (1994) Transgenic expression of IL-10 in pancreatic islet A cells accelerates autoimmune insulitis and diabetes in non-obese diabetic mice. Intern Immunol 6:1927–1936
218. Berman RM, Suzuki T, Tahara H, Robbins PD, Narula SK, Lotze MT (1996) Systemic administration of cellular IL-10 induces an effective specific and long-lived immune response against established tumor in mice. J Immunol 157:231–238
219. Di Carlo E, Coletti A, Modesti A, Giovarelli M, Forni G, Musiani P (1998) Local release of interleukin-10 by transfected mouse adenocarcinoma cells exhibits pro- and anti-inflammatory activity and results in a delayed tumor rejection. Eur Cytokine Netw 9:61–68
220. Vora M, Romero LI, Karasek MA (1996) Interleukin-10 induces E-selectin on small and large blood vessel endothelial cells. J Exp Med 184:821–829
221. Buelens C, De Groote D, Goldman M, Willems F (1996) Differential effects of interleukin-10 on the production of interleukin-12 and interleukin-8 by human dendritic cells generated from peripheral blood. Transplant Proc 28:3255–3256
222. Pang G, Ortega M, Zighang R, Reeves G, Clancy R (1997) Autocrine modulation of IL-8 production by sputum neutrophils in chronic bronchial sepsis. Am J Respir Crit Care Med 155:726–731
223. Becherel PA, Le Goff L, Ktorza S, et al (1995) Interleukin-10 inhibits IgE-mediated nitric oxide synthase induction and cytokine synthesis in normal human keratinocytes. Eur J Immunol 25:2992–2995
224. Cunha FQ, Moncada S, Liew FY (1992) Interleukin-10 inhibits the induction of nitric oxide synthase by interferon-gamma in murine macrophages. Biochem Biophys Res Commun 182:1155–1159
225. Fouqueray B, Boutard V, Philippe C, et al (1995) Mesangial cell-derived interleukin-10 modulates mesangial cell response to lipopolysaccharide. Am J Pathol 147:176–182

226. Betz-Corradin SB, Fasel N, Buchmuller-Rouiller Y, Ransijn A, Smith J, Mauel J (1993) Induction of macrophage nitric oxide production by interferon-gamma and tumor necrosis factor-alpha is enhanced by interleukin-10. Eur J Immunol 23:2045–2048
227. Sunyer T, Rothe L, Jiang X, Osdoby P, Collin-Osdoby P (1996) Proinflammatory agents, IL-8 and IL-10, upregulate inducible nitric oxide synthase expression and nitric oxide production in avian osteoclast-like cells. J Cell Biochem 60:469–483
228. Groux H, Bigler M, de Vries JE, Roncarolo MG (1998) Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells. J Immunol 160:3188–3193
229. Marie C, Pitton C, Fitting C, Cavaillon J-M (1996) Regulation by anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGFβ) of interleukin-8 production by LPS- and/or TNFα-activated human polymorphonuclear cells. Mediators Inflamm 5:334–340
230. Adib-Conquy M, Petit A-F, Marie C, Fitting C, Cavaillon J-M (1999) Paradoxical priming effects of interleukin-10 on cytokine production. Int Immunol 11:689–698
231. Lelievre E, Sarrhouilhe D, Morel F, Preud'homme JL, Wijdenes J, Lecron JC (1998) Preincubation of human resting T cell clones with interleukin-10 strongly enhances their ability to produce cytokine after stimulation. Cytokine 10:831–840
232. Rabbi MF, Finnegan A, Al-Harthi L, Song S, Roebuck KA (1998) Interleukin-10 enhances tumor necrosis factor-activation of HIV-1 transcription in latently infected T cells. J AIDS Hum Retrovirol 19:321–331
233. Arock M, Zuany-Amorim C, Singer M, Benhamou M, Pretolani M (1996) Interleukin-10 inhibits cytokine generation from mast cells. Eur J Immunol 26:166–170
234. Moon TC, Murakami M, Ashraf M, Kudo I, Chang HW (1998) Regulation of cyclooxygenase-2 and endogenous cytokine expression by bacterial lipopolysaccharide that acts in synergy with c-kit ligand and Fce receptor I crosslinking in cultured mast cells. Cell Immunol 185: 146–152
235. Ishimi Y, Miyaura C, Jin CH, et al (1990) IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297–3303
236. Tsujinaka T, Fujita J, Ebisui C, et al (1996) Interleukin-6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin-6 transgenic mice. J Clin Invest 97:244–249
237. Jongen-Lavrencic M, Peeters HRM, Rozemuller H, et al (1996) IL-6 induced anaemia in rats: possible pathogenic implications for anaemia observed in chronic inflammations. Clin Exp Immunol 103:328–334
238. Barber AE, Coyle SM, Marano MA, et al (1993) Glucocorticoid therapy alters hormonal and cytokine responses to endotoxin in man. J Immunol 150:1999–2006