Compensatory anti-inflammatory response syndrome

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Summary
The concept of 'Compensatory anti-inflammatory response syndrome' (CARS) was proposed in 1997 by Roger Bone (1941–1997) to qualify the consequences of the counter-regulatory mechanisms initiated to limit the overzealous inflammatory process in patients with infectious (sepsis) or non-infectious systemic inflammatory response syndrome (SIRS). One major consequence of CARS is the modification of the immune status that could favour the enhanced susceptibility of intensive care patients to nosocomial infections. Indeed, most animal 'two-hit' models illustrate an enhanced sensitivity to infection after a first insult. However, this observation is highly dependent on the experimental procedure. Numerous functions of circulating leukocytes are altered in sepsis and SIRS patients, as well as in animal models of sepsis or SIRS. However, this is rather a reprogramming of circulating leukocytes, since there is not a global defect of the immune cells functions. Furthermore, within tissues, leukocytes are rather primed or activated than immunosuppressed. Thus, CARS may be considered as an adapted compartmentalized response with the aim to silence some acute pro-inflammatory genes, and to maintain the possible expression of certain genes involved in the anti-infectious process.

Keywords
Immunity, monocyte, neutrophil, cytokine, endotoxin, infection, two-hit model

Sepsis and systemic inflammatory response syndrome

The incidence of sepsis continues to rise, and sepsis remains a life threatening event resulting in the death of more than 215,000 US citizens a year (1). In the 1970's it emerged that the more severe form of sepsis, septic shock, was associated with organ failure (2). In 1992, it became obvious that sepsis was associated with a generalized inflammatory reaction in organs remote from the initial insult, and the acronym SIRS for "systemic inflammatory response syndrome" was coined (3). Simultaneously, it was admitted that non-infectious SIRS could be observed in patients with trauma, burns, pancreatitis, hemorrhagic shock, severe surgery and in patients who had been resuscitated after cardiac arrest.

During infection or severe inflammatory insult, microbial molecules or endogenous danger signal molecules and mediators from the host are capable of modulating the homeostasis of the host. Local or systemic inflammatory reactions may be beneficial or deleterious: (i) the struggle against the infectious agent can be overzealous and lead to organ dysfunction (2); (ii) the anti-inflammatory response aimed to dampen the inflammatory process may alter the immune status (4); and (iii) the equilibrium between the procoagulant and anticoagulant status of the host is altered (5). Coagulation becomes activated by circulating endotoxin or bacteria and by some pro-inflammatory cytokines, and a procoagulant state develops in the vascular. This state is tissue factor-dependent (6, 7). Concomitantly, the fibrinolytic system is reduced (8). Indeed, inhibition of activated fibrinolysis predicts microbial infection, septic shock and mortality of febrile patients (9). Disseminated intravascular coagulation (DIC) is a common feature observed in patients with sepsis. Although the potential beneficial effects of coagulation inhibitors have been demonstrated by numerous assays performed in animal models, all of the clinical trials, apart from one, have failed to show a significant benefit concerning survival (10). Treatment with activated Protein C improves survival and other outcome parameters in severe sepsis, but it may not only be linked to its anti-coagulation properties (11).

The present review aims to present the state of the art with respect to the consequences of the infection, the inflammatory response and its anti-inflammatory component on innate immunity and particularly on immune status of circulating leukocytes.
The concept of CARS: an adaptive response

In the 1990’s, sepsis was considered to be associated with an exacerbation of pro-inflammatory mediators as illustrated by the so-called “cytokine storm” (4), and new therapeutic approaches were designed to neutralize these mediators. Simultaneously, it appeared that sepsis was also associated with an enhanced release of anti-inflammatory mediators as shown for soluble tumour necrosis factor receptor (sTNFR) (12), interleukin-10 (IL-10) (13), IL-1 receptor antagonist (IL-1Ra) (14), and transforming growth factor-β (TGFβ) (15). It was then proposed that circulating anti-inflammatory mediators contribute to the body’s normal response to prevent systemic inflammation (16). This concept was in agreement with the words “natura medica-trix” proposed in 1880 by Louis Pasteur about a patient who survived puerperal septicemia. However, the release of anti-inflammatory mediators also appeared to be exacerbated, as illustrated by the strong relationship between high levels of these mediators measured within the bloodstream and poor outcome. Indeed, the plasma of sepsis patients has the capacity to inhibit leukocyte functions, and can be considered as an immunosuppressive milieu (17). In the meantime, there were numerous indications that sepsis and SIRS patients displayed an altered immune status, as assessed by both in vivo (e.g. anergy to skin test antigen [18, 19]), and tests in vitro (e.g. lymphocyte proliferation [20]). Furthermore, the high susceptibility of intensive care patients (ICU) to nosocomial infections has been associated with the occurrence of an alteration of the immune status. Thus, words such as anergy (21), immunodepression (22) and immunoparalysis (23) have been employed to define the immune status of the SIRS patients. Accordingly, the long list of altered properties of immune cells in sepsis and non-infectious SIRS patients led Bone (24) in 1997 to coin a new acronym: CARS for “compensatory anti-inflammatory response syndrome”. Although Bone hypothesized that either SIRS or CARS could predominate in a given patient, and although other authors postulated that CARS follows SIRS in a two-wave process, we rather considered that both events are concomitant (25). In addition, in agreement with Munford and Pugin (16), we rather regard this to be a normal response to limit the systemic inflammatory process. We consider that CARS is not a generalized phenomenon that dampens all immune functions, rather an adaptation depending upon the compartments (i.e. blood vs. tissues), the type of primary and secondary insult, the nature of the studied function, the nature of the produced mediators, and the nature of the leukocytes (Table 1). For example, apoptosis can be either enhanced (lymphocytes, den-
Apoptosis

Apoptosis is a phenomenon that is unevenly observed in sepsis; apoptosis mostly affects lymphocytes in blood (27) and spleen (28), and gastrointestinal epithelial cells (29). Whether endothelial cells undergo apoptosis during sepsis remains a controversial issue (30). Nevertheless, Kuckeburg et al. (31) elegantly demonstrated that the interactions between bacteria-activated platelets and the endothelium may play a key role in the vascular pathology of bacterial sepsis. They showed that endothelial cell apoptosis induced by activated platelets required activation of both caspase-8 and caspase-9, and the production of reactive oxygen species. Regarding monocytes, an increased mitochondrial membrane potential was reported during severe sepsis, but neither circulating monocytes (32) nor spleen macrophage (33) undergo aberrant apoptosis.

The significant protection against cell damage and death in animal models of sepsis, by preventing apoptosis using caspase inhibitors (34, 35) or using transgenic mice overexpressing Bcl2 (36, 37), suggests a key role for this phenomenon in the pathogenesis of sepsis. Apoptosis can cause immunosuppression by two mechanisms: depletion of various immune cells resulting in the loss of key anti-microbial function, and inducing immunosuppressive effects in the surviving cells. High mobility group box-1 (HMGB-1) appears as the key element that links apoptosis with sepsis-mediated mortality (38). HMGB-1 is a nuclear factor that is released by apoptotic and necrotic cells and acts as a late mediator of sepsis (39). Interestingly, HMGB-1 plasma levels in intensive care patients correlated with the disseminated intravascular coagulation score and sepsis-related organ failure (40).

In contrast, the reduced apoptosis of PMNs is also a hallmark of sepsis and SIRS. This may also result from the action of environmental mediators, such as LPS, TNF, granulocyte- and granulocyte-macrophage colony stimulating (G- and GM-CSF), although it can be counter-regulated by IL-10 (41). Interestingly, the pre-B cell colony-enhancing factor (PBEF) produced by PMNs from septic patients functions as an inhibitor of apoptosis within an autocrine loop (42). In addition, the deficit in the release of cathepsin D by PMNs of septic patients could be another concomitant phenomenon that explains their reduced apoptosis, since cathepsin D activates caspase-8 and apoptosis of PMNs (43).

The status of blood neutrophils

Infection is associated with a boost of hematopoiesis. As a consequence, patients with bacterial infections showed higher numbers of circulating myeloid progenitor cells of granulocytopenesis (44). Interestingly, the number of granulocyte-macrophage colony forming cells was higher among younger patients. In parallel, the number of immature neutrophils was increased. However, their identification is difficult, and the percentage in septic patients may vary between 2% and 39% depending upon the studies! (45, 46). In a recent study, phagocytosis by immature neutrophils of septic patients was reported to be lower than that of mature neutrophils from both patients and controls (46).

As illustrated in Table 1, the functions of circulating neutrophils (polymorphonuclear cells, PMN) in sepsis and SIRS patients can be either reduced or enhanced and/or primed. Similarly, the expression of different receptors can be up- or down-regulated. Interestingly, the enhanced expression of CD64 (FcγRI) was shown to correlate with plasma levels of IL-8, severity of sepsis and mortality (47). In the case of chemokine receptors, it was reported that the reduced expression of CXCR2 was associated with a markedly suppressed response to its ligands (48). An impaired intracellular microbial activity has been reported (49). Ex-vivo cytokine (IL-1β, IL-1Ra, and IL-8) production was also reduced after exposure to endotoxin (lipopolysaccharide, LPS) in neutrophils obtained from septic patients compared to healthy controls (50–52). This has been further confirmed after an i.v. administration of LPS in healthy human volunteers whose neutrophils displayed a reduced capacity to produce chemokines upon in-vitro stimulation (53). Nevertheless, the reduced ex-vivo cytokine production by septic neutrophils and non-infectious SIRS patients was observed with some (e.g. LPS) but not all stimuli (e.g. heat-killed Staphylococcus aureus) (54).

However, there are few items that are associated with controversial results. For example, in patients with sepsis, phagocytic activity was reported to be either impaired or increased (54–56). Similarly, generation of reactive oxygen species (ROS) was found to be either down- or upregulated in sepsis (54, 56). These divergent results may reflect the use of different stimuli. For example, Kaufmann et al. (55) reported that hydrogen peroxide production by neutrophils from septic patients could be either reduced (e.g. in response to zymosan), unchanged (e.g. in response to phorbol myristate acetate, PMA, or opsonized zymosan), or enhanced (e.g. in response to tumour necrosis factor, TNF, or formyl methionyl leucyl ester, fMLP). Furthermore, the timing of the recovery of neutrophils after the insult may modify their properties, as suggested by a study in trauma patients for whom the cells were primed and activated in the first 24 hours post injury, but not later (57). Finally, to illustrate the complexity of the phenomenon, one should recall the work by McCall’s group who showed that there are two subsets of neutrophils in patients with acute bacterial infection, one with a normal oxidative response, and another that is primed (58). Interestingly, plasma of sepsis and SIRS patients can modify the functions of PMNs from healthy controls (59–61). Numerous mediators can contribute to the primed status of circulating PMN. Among the mediators that can be found in the plasma of sepsis or SIRS patients, LPS (62), C5a (63), TNF (64), IL-8 (61), IL-18 (65), TREM-1 ligand (66), and platelet activating factor (PAF) (59) have been shown to prime PMNs.

The status of monocytes

As previously mentioned for neutrophils, the nature and the characteristics of circulating monocytes is greatly influenced by the margination and sequestration of activated cells and the boost of hematopoiesis. As shown in a murine model of burn and sepsis, the circulating inflammatory subset (F4/80+ Gr1+) is increased...
post-injury and infection, while the frequency of bone-marrow derived precursors is decreased (67). In human sepsis, the decreased expression of the fractalkine receptor (CX3CR1) on circulating monocytes, further illustrates the presence of different monocytes subsets within the blood stream of septic patients (68). The status of monocytes is modified during sepsis and SIRS as assessed by ex-vivo measurement of oxidative burst, cytokine production or HLA-DR expression at the cell surface (Table 1).

**Oxidative burst**

Oxidative burst and reactive oxygen species are important to fight pathogens and permit their destruction by professional phagocytes. Nitric oxide (NO) is produced in small amounts by constitutive NO synthases, but in large amounts by the inducible NO synthase (iNOS). Oxidative burst in monocytes exposed to PMA is significantly attenuated in septic patients. Inhibition of the oxidative burst and depletion of protein kinase C alpha were correlated in septic patients (69).

**Ex-vivo cytokine production**

The altered ex-vivo cytokine production upon cell-activation has been widely described for circulating monocytes. Monocytes reactivity to LPS has been under particular scrutiny. Upon activation with LPS, monocytes from septic and non-septic SIRS patients display a diminished capacity to release TNF, IL-1α, IL-1β, IL-6 (70), and IL-12 (71). Not only cell machinery is affected, there is also a reduced number of cytokine-producing cells as assessed by flow-cytometry analysis (72). Once again, similar findings were observed in healthy volunteers after LPS exposure (73).

Most interestingly, the impaired capacity of monocytes to produce inflammatory cytokines in response to LPS has been described in numerous clinical settings, including different types of bacterial, viral and parasitic infections, different types of non-infectious SIRS or in patients with a severe organ dysfunction (pancreatitis, heart or liver failure). However, as discussed below, the capacity of monocytes from sepsis or SIRS patients to produce cytokine, can be unchanged or even enhanced when other activators are used instead of LPS, or when other cytokines are studied (IL-1Ra, IL-10, macrophage migration inhibitory factor [MIF]). In addition, different results may be found when studying isolated monocytes versus whole blood, because of the presence of soluble mediators that interfere with monocytes reactivity in the latter case. Thus, there is no global defect of the ex-vivo cytokine production, rather a specific alteration of the production of some pro-inflammatory cytokines in response to some, but not all, stimuli. Therefore, the term ‘reprogrammation’ best characterizes these modifications in leukocyte reactivity (74). In addition, differences can be found between patients, depending on the insult at the origin of the SIRS. For example, the ex-vivo production of TNF in response to LPS is not reduced beyond two days after surgery (75), whereas trauma patients display a long-lasting hyporeactivity several days after their admission (76). Similarly, the ex-vivo production of IL-8 upon LPS activation in whole blood samples was shown to be lower among patients with sepsis as compared to healthy controls, whereas it was unchanged in patients who underwent surgery and cardiopulmonary by-passes (77). The use of anaesthetic drugs before the insult may limit cellular reprogramming following surgical injury, as opposed to trauma or burn. If this holds true, it would imply that neuromediators generated during the insult contribute to cellular reprogramming.

Most studies reported a decreased ex-vivo production of pro-inflammatory cytokines by leukocytes from sepsis or SIRS patients. However, the production of G-CSF was shown to be enhanced in a longitudinal analysis of LPS-activated whole blood samples from ICU patients (78). More surprisingly, the release of MIF, considered as a pro-inflammatory cytokine, was enhanced upon ex-vivo culture without or with different activators of leukocytes of septic patients. This increased MIF production was observed for patients who were not treated with glucocorticoids, whereas the ex-vivo production was similar to controls in patients treated with glucocorticoids (79). When anti-inflammatory cytokines (i.e. IL-1Ra, IL-10) were investigated, no modification or even an enhanced production was reported. We recently observed an enhanced production of IL-10 by monocytes from septic patients in response to both LPS (a Toll-like receptor-4 [TLR4] agonist) and Pam3CysSK4 (a synthetic lipopeptide that is a specific ligand of TLR2) (80). A similar enhanced IL-10 production was observed with circulating leukocytes after surgery or trauma (81). In patients resuscitated after cardiac arrest (RCA), we observed an unaltered production of IL-10 (82). The fact that after LPS-triggering, monocytes can display a reduced production of TNF and an unaltered or even enhanced production of IL-10, further illustrates that the sensing of LPS by monocytes is accompanied by a modification of the intracellular signalling pathways that limits the production of pro-inflammatory cytokines and maintains or favours that of anti-inflammatory ones.

Although the use of highly specific TLR agonists is useful to further understand the alteration of specific signalling pathways within cells from SIRS patients, the response to whole bacteria may represent a more relevant and physiological approach to monitor immune status. For instance, in contrast to LPS and Pam3CysSK4, the production of TNF by isolated monocytes of septic or RCA patients in response to heat-killed *Escherichia coli* or *Staphylococcus aureus* was not diminished when compared to that obtained with cells from healthy donors (80). In whole blood assays, TNF production induced by *S. aureus* was unaltered in trauma and RCA patients, while different results were reported for sepsis (81, 82). In contrast, TNF production in whole blood assays was diminished in sepsis and trauma patients in response to *E. coli* (81, 83). Bacteria can activate monocytes following their interaction with various receptors on the cell surface, but also after phagocytosis. In healthy volunteers and sepsis patients, TNF and IL-10 production in response to *S. aureus* was reduced when phagocytosis was prevented by cytochalasin D. These results suggest that both surface receptors and internal sensors are involved in cytokine production (80). In addition to the surface sensors (TLR2 and TLR4), bacteria can be detected by intracellular sensors. TLR9 is a receptor present in endosomal cavities, which recognizes bacterial DNA. NOD1 is an intracytoplasmic sensor of a peptidoglycan motif and is mainly expressed in Gram-negative bacteria, and NOD2 detects fragments of any bacterial peptidoglycan through their minimal structure, the mu-
ramyl dipeptide (MDP). We showed that NOD1 and NOD2 mRNA expression was similar in the monocytes of healthy controls and patients. This may explain the maintained responsiveness to MDP and whole bacteria that we observed in septic patients (80).

**Gene expression and modifications in intracellular signalings**

Although the microarray technology would have been of interest to address the evolution of gene expression through the course of sepsis, very little has been made so far in isolated monocytes. In human volunteers administered with intravenous endotoxin, the greatest change in mononuclear cell gene expression occurred at 6 hours (439 induced and 428 repressed) (84). A study of gene expression in monocytes revealed that the genes coding for the molecules of the inflammasome were significantly lower in patients with septic shock compared with critically ill patients (85). In polytrauma patients, the pattern of gene expression in monocytes could discriminate between survivors and non-survivors (86).

Several groups, including ours, aimed to decipher the intracellular and molecular mechanisms responsible for the altered responsiveness of monocytes, particularly to LPS. The negative regulation of the LPS-induced TLR4 signalling pathways has been investigated. NF-κB is the main transcription factor required for the expression of the genes coding for inflammatory molecules. NF-κB exists as an active p65p50 heterodimer, whereas its p50p50 homodimer behaves as an inhibitory form. A significant decrease of the ratio between the p65p50 heterodimer and the p50p50 homodimer was reported for monocytes of septic and trauma patients as compared to healthy volunteers (87). The ratio was even lower in non-surviving patients. This observation resembles what was described to occur within mononuclear cell lines rendered tolerant to endotoxin (88). Many molecules have been described to negatively regulate the TLR4 signaling pathways and to contribute to endotoxin tolerance. Interleukin (IL)-1 receptor associated kinase (IRAK)-M prevents the dissociation of IRAK-1 and IRAK-4 from myeloid differentiation 88 (MyD88) and the formation of IRAK-TRAF6 complex, and is a negative regulator of TLR signalling. The so-called “endotoxin tolerance” is significantly reduced in IRAK-M deficient mice (89), which corroborates the observation that ex-vivo LPS-stimulated monocytes from septic patients express IRAK-M mRNA more rapidly than cells from healthy donors (90). Other inhibitory molecules of the TLR pathway have been subject to scrutiny and may play an important role in the adaptive mechanism to inflammatory processes. Toll interacting protein (Tollip) is an adaptor protein that potently suppresses the activity of IRAKs after TLR activation (91). Suppressor of cytokine signaling-1 (SOCS-1) is one of eight members of a family involved in the negative regulation of cytokine signal transduction pathways, particularly the JAK/STAT pathway (92). An LPS-inducible splicing variant of MyD88, termed “MyD88 short” (MyD88s), is defective in its ability to induce IRAK phosphorylation and behaves as a dominant-negative inhibitor of LPS-induced NF-κB activation (93). Single immunoglobulin IL-1 receptor-related...
molecule (SIGIRR), a member of the TLR/IL-1R superfamily, is a negative modulator of the signalling induced by IL-1 or LPS (94). Finally, ABIN3, a member of the A20-binding molecules family, has been shown to be upregulated by LPS or TNF, and to behave as an inhibitor of TLR4 signalling (95). The contribution of these molecules has recently been studied in sepsis. Figure 1 shows the molecules that are upregulated in monocytes from septic patients. These molecules include SIGIRR, MyD88s and IRAK-M. More recently, we also showed that the expression of mRNA coding for ABIN3 was increased in the monocytes of septic patients, and that this expression was normalized after in vivo treatment of septic shock patients by corticosteroids, whereas this treatment had no effect on MyD88s and SIGIRR (96). Finally, an impaired activation of extracellular signal-regulated kinase (ERK) was reported in LPS-activated monocytes from septic patients but not from non-infectious SIRS (97). The increased expression of these inhibitors of the NF-κB pathway in monocytes of sepsis and SIRS patients most probably contributes to the altered pro-inflammatory response to LPS and other microbial products.

**HLA-DR expression**

The reduced expression of HLA-DR molecule on monocytes is a hallmark of sepsis and SIRS (98, 99), and is also observed following LPS injection in healthy human volunteers (100). The downregulation is more pronounced in superinfected trauma patients and is associated with a poor outcome (98). Decreased MHC Class II expression is universally described in sepsis but only its evolution over time can distinguish between survivors and non-survivors, the decreased HLA-DR expression being present in the non-survivors group (99, 101). The decreased HLA-DR expression is found for the number of cells expressing this molecule, as well as for the amount of HLA-DR expressed per cell (102). As expected, the decreased expression of MHC Class II antigen results in an altered antigen presentation capacity (103). Low HLA-DR expression is associated with an increased risk of secondary bacterial infections (104), probably due to a less potent antigen presentation that would not allow an efficient adaptive immunity.

**The status of blood lymphocytes**

It is often suggested that immunosuppression is mainly observed for Th1 cytokines (IL-2, IFNγ) while production of the Th2 type would be upregulated. In fact, this concept may hold true for animal models of SIRS or sepsis (105), but it is not easy to demonstrate in humans because studied lymphocytes are not derived from the same compartments (mice spleen vs. human blood). Indeed, both Th1 and Th2 cytokines were decreased after ex vivo lymphocyte stimulation by T cell mitogens in patients with sepsis or after cardiopulmonary bypass surgery (106). Furthermore, an identicaly altered production for both Th1 and Th2 populations was reported in patients after successful RCA (82) and in trauma patients (107).

Rapid mobilization and subsequent redistribution of leukocytes occurs during sepsis and SIRS. The nature of the leukocytes found within the blood stream reflects: (i) the disappearance of activated cells that bind to endothelium and migrate towards inflammatory tissues, (ii) the enhanced apoptosis of lymphocytes (27, 28) and the delayed apoptosis of neutrophils, and (iii) the boost of hematopoiesis that leads to the release of freshly produced leukocytes from the bone marrow. These events lead to markedly different circulating leukocyte subpopulations as compared to healthy controls. B and T-lymphopenia, and neutrophilia are a hallmark of sepsis that can be mimicked in human volunteers receiving a bolus of LPS. Lymphopenia affects both CD4+ and CD8+ populations, and among CD4+ cells, the increased percentage of circulating regulatory T-cells (Treg, CD4+ CD25+) was indeed due to a decrease of the CD4+ CD25− lymphocyte population (108).

**The status of leukocytes within tissues**

As expected, the immune status of leukocytes present within inflammatory foci display a different status than the cells isolated from the blood stream; this is known as compartmentalization (4). The nature of the insult (e.g. burn, haemorrhage, trauma, peritonitis, etc.), the cellular composition of each compartment (e.g. nature of resident phagocytes, nature of endothelial cells), and its micro-environment (e.g. local presence of GM-CSF in the lungs, low levels of arginine in the liver, release of endotoxin from the gut), and leukocyte recruitment, have a great influence on local inflammation and on tissue injury. Experimental animal models have shown that neutrophils do contribute to lung injury after haemorrhage and infection (109). Abraham et al. clearly demonstrated in murine models of endotoxemia and hemorrhagic shock that lung-derived neutrophils displayed activation of transcriptional regulatory factors and intracellular kinase and an increased expression of inflammatory cytokines, whereas these activations were not observed in blood neutrophils (110, 111). In humans, the most convincing experiments were conducted by Coldren et al. (112) in volunteers exposed to endotoxin by bronchoscopic instillation. The authors reported a dramatic gene expression difference between air space and circulating neutrophils. These results suggested that neutrophils sequestered in the lung become fundamentally different from those resident in the circulation. In addition, PMNs from inflammatory foci appeared to be poorly sensitive to anti-inflammatory signals such as IL-10 (113, 114) or corticoids (115). Still in the lung, alveolar macrophages also display numerous signs of activation as observed in animal models of haemorrhagic shock or sepsis (116, 117). Interestingly, we showed that murine alveolar macrophages were resistant to the induction of endotoxin tolerance, in contrast to mononuclear phagocytes derived from other compartments (118). In human acute respiratory distress syndrome, alveolar macrophages release enhanced levels of chemokine and cytokine and display enhanced transcription factor activation (119–121). Similarly, alveolar macrophages harvested from patients after cardiopulmonary bypass produced higher levels of TNF and IL-1 than before cardiopulmonary bypass when stimulated in vitro (122).

Although most studies reveal an activated status of leukocytes within inflammatory foci, these cells can still display some inhibitory activity. For example, immature myeloid cells or monocytes isolated at the burn-site of skin tissue, can exert local suppressive action, such as the capacity to inhibit antimicrobial β-defensin production by keratinocytes (123).
### Mediators Produced during Sepsis that Affect Immune Status of Monocytes / Macrophages

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<tr>
<th>Mediators</th>
<th>Properties on Monocytes / Macrophages</th>
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<tr>
<td><strong>Cytokines</strong></td>
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<td>IL-10</td>
<td>Inhibits cytokine and chemokine production</td>
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<td>Inhibits macrophage cytotoxicity</td>
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<td>Decreases microbicidal activity</td>
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<td>Suppresses release of reactive oxygen intermediates</td>
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<td>Induces sequestration of HLA-DR molecules</td>
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<td></td>
<td>Stimulates FcR (CD16, CD64), TNFR I &amp; II, and CCR5 expression</td>
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<td>Inhibits LPS-induced tissue-factor expression and procoagulant activity</td>
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<td>Inhibits monocyte adhesion to endothelial cells</td>
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<td>Up-regulates the expression of the S100 protein</td>
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<td>Modulates apoptosis</td>
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<td>TGFβ</td>
<td>Inhibits cytokine and chemokine production</td>
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<td>Inhibits macrophage cytotoxicity</td>
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<td>Decreases microbicidal activity</td>
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<td>Increases PGE2 production</td>
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<td>Suppresses nitric oxide (NO) release and H₂O₂ production</td>
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<td>Down-regulates procoagulant activity and tissue factor expression</td>
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<td><strong>Lipid mediators</strong></td>
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<td>PGE2</td>
<td>Inhibits TNF and chemokine production</td>
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<td>Up-regulates the synthesis of IL-6 and IL-10</td>
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<td>Inhibits phagocytosis</td>
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<td>Down-regulates the expression of CCR5, MHC Class II molecules</td>
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<td>Decreases iNOS synthesis</td>
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<td>Inhibits myelopoiesis</td>
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<td><strong>Neuromediators and hormones</strong></td>
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<td>Epinephrine</td>
<td>Suppresses LPS-induced TNF and NO production</td>
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<td>Enhances LPS-induced IL-10 production</td>
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<td>Prevents LPS-induced down-modulation of TNF receptors</td>
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<td>Depresses antibody-dependent phagocytosis</td>
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<td>Acetylcholine</td>
<td>Inhibits pro-inflammatory cytokine and HMGB-1 release</td>
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<td>Vasoactive intestinal peptide (VIP)</td>
<td>Inhibits LPS-induced cytokine &amp; chemokine production</td>
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<td>Inhibits respiratory burst and iNOS expression</td>
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<td>Inhibits phagocytosis and chemotaxis</td>
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<td>Down-regulates CD80, CD86 expression</td>
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<td>Favour IL-10 and IL-1RA production</td>
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<td>Pituitary adenylate cyclase activating peptide (PACAP)</td>
<td>Inhibits the production of pro-inflammatory cytokines</td>
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<td>Stimulates the production of anti-inflammatory cytokines</td>
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<td>Downregulates CD80, CD86 expression</td>
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<td>Serotonin</td>
<td>Inhibits LPS-induced TNF production</td>
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<td>α-melanocyte stimulating hormone (α-MSH)</td>
<td>Suppresses TLR4-induced signaling</td>
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<td>Stimulates IL-10 production</td>
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<td><strong>Stress molecules</strong></td>
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<td>Heat shock proteins</td>
<td>Inhibit LPS-induced production of cytokines</td>
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<td>Glucocorticoids</td>
<td>Inhibit cytokine production</td>
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<td>Inhibit free radical production</td>
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<td>Inhibit PGE2 production</td>
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<td>Inhibit chemotaxis</td>
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<td>Induce IL-10 production</td>
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<td>Induce IκB production</td>
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<td><strong>Others</strong></td>
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<tr>
<td>Ubiquitin</td>
<td>Inhibits LPS-induced production of TNF</td>
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Table 2: Inhibitory mediators produced during sepsis that affect immune status of monocytes / macrophages.
The role of circulating mediators

Numerous mediators can dampen the inflammatory response and limit the capacity of leukocytes to produce pro-inflammatory cytokines in response to LPS (Table 2). The fact that the response of whole blood samples of septic shock patients is enhanced in samples collected after plasma filtration and adsorption strongly suggests that blood leukocytes are bathing within an inhibitory milieu (124). The presence of deactivating or immunosuppressive agents within the blood stream most probably contributes to the hyporeactivity of circulating leukocytes. In the late 1970's it was reported that sera of burn patients were able to suppress the proliferative response of normal cells (125). More recently, Prins et al. (126) showed that sera from septic patients had the capacity to downregulate the TNF production by activated monocytes from healthy donors. Also, deactivating properties were reported for sera from trauma patients (127) and after cardiopulmonary bypass (128). Similarly, the plasma obtained from successfully resuscitated cardiac arrest patients was able to blunt the TNF production by leukocytes from healthy controls after LPS exposure (82, 129). The fact that "septic plasma" behaves as an immunosuppressive milieu (17) is illustrated in human volunteers by the capacity of endotoxin to induce plasma inhibitors (130). The effects of septic or SIRS plasma are not limited to leukocytes and their capacity to induce cardiac myocyte apoptosis and to impair mitochondrial function have also been reported.

Some of these plasma factors are able to neutralize endotoxin. Those include soluble CD14 (131) and LPS-binding protein (LBP) (132) that favours the transfer of LPS to lipoproteins known to neutralize endotoxin (133). High density lipoproteins (HDL) and other plasma lipoproteins can bind and neutralize the bioactivity of Gram-negative bacterial LPS (134) and Gram-positive bacterial lipoteichoic acid (LTA) (135). MD-2 is a soluble protein that associated to TLR4 forms the receptor for LPS. Soluble MD-2 has been detected in the plasma of patients with severe sepsis or septic shock, and in lung edema fluids from patients with acute respiratory distress syndrome (ARDS) (136). Similar to sCD14, sMD-2 may enhance the reactivity of TLR4-positive epithelial cells towards LPS, whereas it would diminish expression of HLA-DR molecules onto monocytes, by inducing its intracellular sequestration. The neutralization of interleukin-10 in a model of CLP post-burn injury was able to restore alveolar macrophages function post-peritonitis (141).

Animal models have allowed the identification of cell subsets that can contribute to the alteration of the immune status. Among IL-10 producing cells, Treg contribute to some of the aspects of the sepsis-induced lymphoid immune suppression since depletion of CD4+ CD25+ cells in vivo before CLP markedly restored CD4+ CD25+ proliferative capacity and Th1 cytokine release, without altering plasma proinflammatory cytokine levels. However, the depletion of CD25+ cells before induction of sepsis did not alter septic mortality (142). In contrast, in the same CLP model, adoptive transfer of Treg stimulated in vitro in both prevention and therapeutic models, significantly increased peripheral TNFα production, and improved bacterial clearance and survival (143). Interleukin-10 is also expressed by heterogeneous, immature, predominantly myeloid progenitors cells that are GR-1+ CD11b+. This population is dramatically increased and remains elevated in the spleen, lymph nodes, and bone marrow during polymicrobial sepsis (144). GR-1+ cell depletion in vivo prevents both the sepsis-induced increase of Th2 cell-dependent and decrease of Th1 cell-dependent antibody production. Thus, GR-1+CD11b+ cells contribute to sepsis-induced T cell suppression and favour Th2 polarization (144).

IL-6 has also been shown to be protective. Using IL-6 KO mice, it was reported that IL-6 was protective in an experimental endotoxic shock model (145), and against coagulatory and haemostatic disturbance and subsequent pulmonary haemorrhage induced by bacterial endotoxin, at least partly, via the modulation of proinflammatory processes (146). Indeed, IL-6 can favour the release of IL-10, IL-1 receptor antagonist, soluble TNF receptor and cortisol (147, 148). It is unknown whether this last network linking IL-6 with cortisol could involve IL-6 with the alteration of the immune status.

IL-10 release can be favoured by the action of catecholamines that are known to contribute to the altered responsiveness of circulating leukocytes. Indeed, in a murine haemorrhagic shock model, β-adrenoceptor blocking allowed a partial restoration of the responsiveness of blood leukocytes to LPS in terms of TNF production (149). Similarly, the mortality due to spontaneous bacterial infection occurring after stroke was dramatically reduced by the administration of β-adrenoceptor blockers (150). Corticosteroids and catecholamines both individually and cooperatively induce a shift of T cells cytokine balance (151), they reduce Th1 and favour Th2 type cytokine production. The effect is mediated through the inhibition of IL-12 production by monocytes (152), but also by a direct effect on Th1 cells (153). Many other neuromediators could be responsible for the reduced reactivity of circulating leukocytes (154); this is particularly the case of acetylcholine (155). Other deactivating agents, such as heat shock proteins, ubiquitin, ligand of TREM-2 or prostaglandins, possibly contribute to the alteration of the immune status. However, there is scant evidence which links these mediators with the observed reduced ex-vivo cytokine release in sepsis or SIRS (156).

The relationship between CARS and the occurrence of secondary infections

Some studies have indicated a correlation between the severity of the alteration of the immune status and an increased probability of developing sepsis among ICU patients (49, 157–159). Similarly, numerous “two hit” animal models have suggested that following a first insult, an enhanced susceptibility to infection oc-
curs. For example, after trauma-haemorrhage (160), acute pancreatitis (161), subcutaneous inflammation (162), or sterile laparotomy (163), an enhanced susceptibility to peritonitis and to Escherichia coli, Pseudomonas aeruginosa or Staphylococcus aureus infections has been reported. Similarly, a first infection, usually a peritonitis induced either by i.p. injection of E. coli, or by CLP, renders the animals more sensitive to a secondary bacterial of fungal lung infection (164–166). Most impressive was the spontaneous occurrence of septicemia and pneumonia in a mouse model of focal cerebral ischaemia (150). The role of IL-10 in the increased susceptibility of animals to a secondary infection has been suggested by the beneficial effects of the neutralization of interleukin-10 in a model of peritonitis post-burn injury (167). In a severe pancreatitis model, it was shown that the serum of the SIRS animals could transfer the enhanced susceptibility to a secondary peritonitis. In this model, the neutralization of the CCL2 chemokine (MCP-1) present in the serum prevented the deleterious effect of serum transfer (168). The authors also showed that PMNs from SIRS animals favoured the production of IL-4 and IL-10 by activated T-lymphocytes and that this phenomenon was CCL2-dependent (169). More recently, an elegant study from the same team convincingly demonstrated that norrepinephrine-treated neutrophils could decrease the resistance of mice to infection (CLP) (170).

All these observations and models tend to support the concept that CARS contributes to the occurrence of secondary infections. However, this concept might be a little too simplistic. The hyporeactivity of leukocytes reported in sepsis and SIRS has sometimes been compared to the phenomenon of endotoxin tolerance (97, 129). However, the authors have demonstrated that the induction of endotoxin tolerance in mice enhances their resistance to fungal and bacterial infection, and to peritonitis (171–173). Another fascinating observation was reported by Takhashi et al. (174). Using two different models, thickness burn injury and pancreatitis, they showed that if the first insult was mild, the resistance to a secondary infection (CLP) was increased, whereas, if the first insult was severe, the mortality to the secondary bacterial infection was higher. Concomitantly, they showed that the anti-bacterial activity of peritoneal macrophages was higher in mice, which underwent a first mild insult than in control mice, and lower in mice, which underwent a first severe insult. Another two-hit model showed the opposite of the usual observation: Lederer’s group (175) reported that after a 25% total body surface area burn injury, the mice displayed an enhanced resistance to a E. coli peritonitis associated with an enhanced presence of microbicidal neutrophils in the peritoneal cavity, and a faster clearance of bacteria. As pointed out by these authors, many parameters may have influenced their results. Particularly, they used a single pathogen model of Gram-negative peritonitis instead of the classical CLP used by most investigators to induce peritonitis. Furthermore, they identified that the delay between the first insult and the secondary infection had an important influence on the observation. Similarly, Männel’s group illustrated that depending upon the nature of the first insult and the nature of the second hit, the mice could be either protected or sensitized (176).

To conclude, we consider that CARS is an adapted response to dampen an overzealous inflammatory response. As we have previously discussed (177), this phenomenon is not a global defect of the immune status of circulating cells, rather an adapted reprogramming of leukocytes. It has been suggested that it contributes to a compartmentalized silencing of acute proinflammatory genes (178). Interestingly, this is in agreement with the recent analysis of endotoxin tolerance which showed that during repeated exposure to LPS, one class of gene (tolerable) including inflammatory cytokines was transiently silenced to prevent pathology-associated excessive inflammation, while a second class of genes (non-tolerizable) including anti-microbial effectors, remained inducible to protect the host from infection (179).

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