Review Article

The nonspecific inflammatory response to injury

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Purpose: The role of the nonspecific inflammatory response in causing injury related to surgery has become better understood over the last decade. There are complex interactions between neutrophils, cytokines and nitric oxide metabolites that may cause organ injury following surgery. The purpose of this review is to summarize some of the processes causing injury through these nonspecific pathways.

Methods: A review of the medical and anaesthetic literature related to inflammation, neutrophils and pro-inflammatory cytokines were performed using Medline. Bibliographies of relevant articles were searched and additional articles were then selected and reviewed.

Results: Pro-inflammatory cytokines, such as tumour necrosis factor, are released in response to a variety of noxious stimuli (e.g. burns, sepsis, or CABG surgery). These cytokines cause activation of neutrophils with increased up-regulation of adhesion complexes on neutrophils and vascular endothelium. Nitric oxide synthase activity is also increased with a resultant increased production of nitric oxide. The increased nitric oxide concentration in the presence of superoxide free radicals secreted by activated neutrophils forms peroxynitrite, a more reactive and toxic molecule. Once this process is initiated, diffuse organ injury can result. Although some information related to specific anaesthetics is available, firm recommendations related to clinical practice cannot be made.

Conclusions: There is a complex interplay of inflammatory mediators that can cause injury. Although specific clinical applications for manipulating these pathways are not yet generally available, this area holds promise to develop new techniques to improve outcomes following surgery.

Objectif : La connaissance du rôle de la réaction inflammatoire non spécifique, causant une atteinte liée à la chirurgie, s'est développée pendant la dernière décennie. Il y a des interactions complexes entre les polynucléaires neutrophiles, les cytokines et les métabolites de l'oxyde nitrique qui peuvent entraîner une lésion organique à la suite d'une chirurgie. Le but de cette revue est de résumer quelques-uns des procédés mis en cause dans les lésions survenant selon ces interactions non spécifiques.

Méthode : Une revue de la documentation médicale et anesthésique sur l'inflammation, les polynucléaires neutrophiles et les cytokines pro-inflammatoires a été faite à l'aide de Medline. Nous avons consulté les bibliographies des articles pertinents et nous avons alors choisi et revu des articles supplémentaires.

Résultats : Les cytokines pro-inflammatoires, comme le facteur nécrosant des tumeurs, sont libérées en réaction à une variété de stimuli nocifs (brûlures, maladie infectieuse ou pontage aorto-coronaire). Ces cytokines provoquent une activation des polynucléaires neutrophiles accompagnée d'une régulation positive accrue des complexes adhésifs sur les polynucléaires neutrophiles et sur l'endothélium vasculaire. L'activité de la NO-synthase est aussi augmentée, ce qui résulte en une production accrue d'oxyde nitrique. L'augmentation de la concentration d'oxyde nitrique en présence des radicaux libres superoxydes, sécrétés par les polynucléaires neutrophiles activés, forme le peroxynitrite, une molécule plus réactive et plus toxique. Une fois ce processus enclenché, une lésion organique diffuse peut survenir. Bien que des informations concernant des anesthésiques spécifiques soient accessibles, des recommandations fermes liées à la pratique ne peuvent être faites.

Conclusion : Il existe une interaction complexe des médiateurs inflammatoires pouvant causer des lésions. Quoique les applications cliniques spécifiques de la manipulation de ces voies métaboliques ne soient généralement pas encore disponibles, cela constitue un domaine prometteur de développement de nouvelles techniques pour améliorer la situation postopératoire.

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I General background

It is not just the severity of an environmental insult that determines the extent of the resultant injury but, rather, the injury occurs within the context of the immune response to insult. Over the last decade there has been increasing understanding of how the presence of an insult is communicated to the various effector cells within the body involved in the immune response. The immune response, in turn, includes an innate nonspecific response as well as an acquired specific response.

In broad terms, following a noxious insult there is an initial response mediated by neutrophils and macrophages (Figure 1). The macrophage response includes the release of a variety of soluble mediators (cytokines) which modulate other parts of the immune response with subsequent involvement by other components of the immune system e.g. natural killer (NK) cells and T helper lymphocytes. These cytokines alter distant cells in a variety of ways including: 1) directing a T lymphocyte response; 2) inducing enzyme production in distant sites (e.g. nitric oxide); and 3) altering cell surface adhesion molecules. This complex interplay of mediators and effectors will be reviewed with emphasis placed upon the roles of selected cytokines, neutrophils and nitric oxide. This review will primarily focus upon the non-specific immune response and its relevance to clinical outcomes following surgical, anaesthetic or intensive care interventions. It is not intended to be all-inclusive

but, rather, to give a brief overview of the main events leading to organ injury.

A Cytokines and neutrophil function

To achieve an effective host defence, communications must be established among distant sites. The role of cytokines as messengers of the immune response has been recently reviewed.¹ Cytokines are low molecular weight proteins that are potent at low concentrations. After binding to specific cell surface receptors the cytokines initiate multiple effects, some of which include synthesis of other cytokines. Cytokine release can occur in phases with an initial local release of IL-1 and TNF α and then a subsequent secondary more distant cytokine release.² Cytokines are broadly classified as: 1) growth factors (e.g. PDGF, TGF- β); 2) modulators of leukocyte chemotaxis, also known as chemokines (e.g. IL-5, IL-8); 3) modulators of lymphocyte function (e.g. IL-2, IL-4); 4) modulators of an inflammatory response (e.g. IFN- γ , IL-1, IL-6 and TNF α). The pro-inflammatory cytokines, typified by TNF, effectively prime the body's defences in a non-specific manner. Other cytokines (e.g. TGF- β , IL-4 or IL-10) down-regulate this inflammatory response.

Tumour necrosis factor α can cause endothelial injury directly³ or secondarily through its interactions with neutrophils.⁴ Neutrophils become nonspecific-

FIGURE 1 Illustrates the nonspecific inflammatory response to an insult. The early pro-inflammatory cytokine response causes increased neutrophil adhesion to endothelial surfaces. Smooth muscle and other cells are stimulated to increase production of nitric oxide. In the presence of increased superoxide (O_2^-) this then results in formation of a peroxynitrite ($ONOO^-$) which may cause further cellular damage.

FIGURE 2 Illustrates the calcium-independent nitric oxide synthase (iNOS) production of nitric oxide (NO). Pro-inflammatory cytokines cause increased expression of iNOS that then catalyzes the production of NO from L-arginine (L-ARG). NO diffuses to vascular smooth muscle and other cells where it stimulates soluble guanylate cyclase (GC-S). This in turn increases intracellular cyclic guanylate monophosphate (cGMP) and, depending upon cell type, causes vascular smooth muscle relaxation, inhibition of platelet adhesion or inhibition of neutrophil adhesion. Glucorticoids can prevent the induction of iNOS.

ly activated following exposure to pro-inflammatory cytokines. They demonstrate increased phagocytosis, increased respiratory burst activity and degranulation.⁵ Approximately 30-90 min following exposure to TNF α , neutrophils produce cytotoxic substances such as hydrogen peroxide.⁶ Finally, exposure to TNF α results in enhanced neutrophil adhesiveness⁷ likely through increased neutrophil expression of cell surface adhesion complexes.⁸ Besides activating neutrophils, cytokines (i.e. IL-8) also modulate neutrophil migration (reviewed by Strieter *et al.*,⁹). IL-8 is a potent neutrophil and lymphocyte chemoattractant that can be isolated from peripheral blood monocytes as well as endothelial cells, fibroblasts and neutrophils. These cells produce IL-8 in response to signalling from other pro-inflammatory cytokines. Although increased local IL-8 production recruits neutrophils to specific sites, once neutrophils have become activated, they may indiscriminately injure other tissues.¹⁰ This indiscriminate injury may be the mechanism underlying syndromes such as ARDS or multiple system organ failure (MSOF) associated with sepsis.

B Neutrophil and endothelial adhesion complexes

Neutrophils must adhere to vascular endothelium to cause organ injury. There are several discrete steps involved with the production of a neutrophil-mediated injury (reviewed by Ward *et al.*¹¹ and by MacNee and Selby,¹²). The adhesion process is a complex phenomenon requiring interactions between endothelial and neutrophil surface adhesion complexes (reviewed by

Frenette and Wagner,^{13,14}). This process has been well described within the lung, a common target organ for injury. The first step in the lung microvasculature is transient retention of neutrophils by purely mechanical factors i.e. neutrophils are large enough to occlude the microvessels.¹⁵ Specific adhesion molecules then become important in determining whether the neutrophils will continue their transit through the vasculature or whether they will more firmly adhere to the endothelium. Neutrophils express L-selectin, a cell surface glycoprotein, which mediates a low-affinity rolling interaction with E-selectin, a molecule found on the endothelial surface (reviewed by Lasky,¹⁶). A different family of cell surface adhesion glycoproteins, CD11/CD18, is responsible for the tight adhesion of neutrophils with the intercellular adhesion molecules (ICAMs) found on the endothelial surfaces. Together CD11/CD18 and ICAMs mediate a tight adhesion of neutrophils to endothelial surfaces and their subsequent extravasation into the interstitium. Expression of these adhesion complexes both on the endothelial surface (ICAMs and E-selectins) and on the neutrophil surface (L-selectin and CD11/CD18) is increased by pro-inflammatory cytokines.¹⁷ There is a differential organ expression of endothelial adhesion molecules in response to pro-inflammatory cytokine signalling with organs such as the lung showing increased E-selectin expression.¹⁸ These types of differences in expression of adhesion complexes may explain some of the propensity for an organ-specific injury following such systemic insults as sepsis.

TABLE Effects of anaesthetics upon cytokine production

<i>Study</i>	<i>Surgery</i>	<i>Limitation</i>	<i>Drug or technique</i>	<i>Effect</i>
Taylor <i>et al.</i> ⁵⁵	Pelvic	Sample small (n = 16) Insufficiently powered	3 or 15 ug/kg fentanyl supplementing halothane	No difference between groups in IL-6
Crozier <i>et al.</i> ⁵⁶	Hysterectomy	Sample small (n = 16)	(1)alfentanil and propofol compared to (2) fentanyl, methohexitone and isoflurane	Greater and earlier release of IL-6 in (2)
De Jongh <i>et al.</i> ⁵⁷	Vaginal delivery and Caesarean Section	no randomization	epidural compared with general	IL-6 higher in parturients who has epidural
Jameson <i>et al.</i> ⁵⁸	Hysterectomy	Sample small(n = 16)	etomidate compared with thiopentone induction	IL-6 higher with etomidate
Norman and Fink ⁵⁹	Abdominal aortic replacement	Sample small(n = 39)	general and postoperative patient controlled morphine compared with combined regional with bupivacaine/ general and postoperative epidural morphine	IL-1b,IL-6, TNF not different between groups
Moore <i>et al.</i> ⁶⁰	Hysterectomy	Sample small(n=16)	General compared to general /extradural	No difference between groups in IL-6

C Nitric oxide, peroxynitrite and free radicals

Nitric oxide (NO) is an endogenous mediator which helps maintain the vasodilator tone of the systemic and the pulmonary vasculature.¹⁹⁻²¹ In addition, NO plays an important role in regulating cell-cell contact by inhibiting platelet aggregation as well as inhibiting platelet and neutrophil adhesion to endothelial cells (reviewed by Radomski²²). The NO synthase (NOS) family of isoenzymes converts L-arginine to NO and L-citrulline, a largely inactive co-product of this reaction.²³ Under physiological conditions, the vascular endothelial cells express a calcium-dependent isoform (eNOS) that releases small (pmol) quantities of NO in response to shear stress and receptor stimulation (reviewed by Moncada²⁴). The eNOS is distinct from a calcium-independent inducible isoform (iNOS) which is expressed *de novo* in endothelium and macrophages by endotoxin and pro-inflammatory cytokines (Figure 2) and generates NO in high (nmol) quantities²⁵ (Radomski *et al.*, 1990). The eNOS plays a crucial role in physiological regulation of blood flow and cell adhesiveness.²² Increased expression of iNOS leads to the formation of excessive amounts of NO which in turn causes hypotension.²⁶⁻²⁸ It is possible to inhibit NOS activity in a nonspecific manner using analogues of L-arginine such as L-NMMA²⁹ or to more specifically inhibit the iNOS isoform using an analogue such as aminoguanidine.³⁰

The cellular injury associated with excessive NO production may, in fact, be due to formation of peroxynitrite (ONOO⁻), a product of the reaction between NO and superoxide.²² Following an insult such as endotoxin administration³¹ or direct neutrophil activation,³² ONOO⁻ production is markedly increased. Peroxynitrite, in turn, causes such diverse phenomena as vascular hyporeactivity,³³ platelet dysfunction³⁴ or surfactant dysfunction.³⁵

Besides ONOO⁻, other toxic, highly reactive molecules (e.g. superoxide, hydrogen peroxide, and hydroxyl radical) are released which aggravates organ injury (reviewed by Goode and Webster³⁶). Free radical production is elevated following diverse insults such as sepsis,³⁷ cardiopulmonary bypass³⁸ or burns.³⁹ There is also evidence that free radical scavengers decrease injury following these insults.⁴⁰⁻⁴³ However, many free radical scavengers are limited by their inability to access the cleft between the adherent neutrophil and the endothelial surface where these toxic products are released.⁴⁴ Thus, as neutrophils are recruited to endothelial surfaces, they release a variety of toxic products that may then directly cause cell death and organ injury.

D The role of anaesthesia

In human studies it is difficult to isolate the role of anaesthetic agents from that of the direct surgical trauma as the cause of nonspecific immunological changes. There is more information on the isolated effects of anaesthetics in animal models. Anaesthesia alone (halothane/isoflurane) or anaesthesia with surgery both produce decreases in basal natural killer (NK) cell cytotoxicity and enhancement of NK activity by interferon.^{45,46} Stanley *et al.*,⁴⁷ reported reduction in human neutrophil chemotaxis and random motion induced by halothane, enflurane, or morphine. A rapidly reversible depression of granulocyte function was also described with moderate concentrations of enflurane⁴⁸ and, more recently, with halothane.⁴⁹ Thiopentone, pentobarbitone, ketamine, halothane but not methoxyflurane depress the neutrophil accumulation within veins after intraperitoneal TNF α injection.⁵⁰ Finally, Duncan *et al.*,⁵¹ demonstrated that halothane increased mortality in a murine model of sepsis. The clinical importance of these observations is not clear.

Anaesthesia also modifies the cytokine response associated with a surgical stress. In a porcine model, prolonged anaesthesia (two hours) with ketamine and pentobarbitone blunted any increases of IL-6.⁵² Thiopentone anaesthesia depresses IL-2 but not IL-4 antigen-stimulated synthesis.⁵³ Monocytes and granulocytes normally show a hyperstimulation on exposure to cardiopulmonary bypass plasma and pretreatment with morphine can diminish this response.⁵⁴ Despite these studies into basic mechanisms, what combination of drugs or techniques that improves postoperative outcomes is still not known. Neuro-axis blockage or use of specific anaesthetic agents has been attempted to modify the nonspecific immunological response to surgery. To control better for confounding variables, these interactions and outcomes have been evaluated in studies restricted to a single type of elective surgical procedure. Even these human studies have not shown consistent clinically applicable results (Table⁵⁵⁻⁶⁰).

II Specific disorders

A Cardiopulmonary bypass

Hall *et al.*,⁶¹ recently reviewed the systemic inflammatory response to cardiopulmonary bypass (CPB). Cardiopulmonary bypass is associated with the release of a host of inflammatory mediators but the clinical importance of any single mediator remains uncertain.⁶² Both IL-6 and TNF α are increased considerably following coronary artery bypass grafting (CABG) surgery⁶³ and these cytokines can cause

increased iNOS activity.⁶⁴ As well, CPB has been shown generally to increase myocardial iNOS activity.⁶⁵ Following CABG surgery a diffuse multi-organ injury can occur with evidence of brain dysfunction,⁶⁶⁻⁶⁸ heart dysfunction,⁶⁹⁻⁷¹ and lung dysfunction.⁷²⁻⁷⁴

Hare *et al.*,⁷⁵ have recently reviewed the roles of eNOS and iNOS in modulating cardiac contractility. Either excessive or deficient generation of NO appears to be detrimental for heart function. Indeed, while the administration of NO donors to replace deficient myocardial NO improved cardiac recovery following ischaemia,⁷⁶ blocking the excessive iNOS-derived myocardial NO also reduced the associated reperfusion injury.⁷⁷ In fact, cytokine-induced decrements of cardiac function appear to be related to increased myocardial iNOS⁷⁸ that in turn results in a damaging increase in peroxynitrite production.⁷⁹ Thus, there appears to be a delicate balance of NO bioactivity necessary for optimal cardiac performance following cardiac injury. Besides NO, alterations in granulocyte function have also been shown to be involved in the cardiac impairment associated with CPB. Inhibition of CD11/CD18 expression⁸⁰ or of CD11/CD18 function⁸¹ has also been shown to improve cardiac function following CPB. Thus, in summary, during CABG there is production of inflammatory mediators, which includes pro-inflammatory cytokines, NO and ONOO⁻. These mediators then cause a diffuse organ injury including impairment of brain, lung, and heart function.

B Intra-operative injury

The role of inflammation in worsening organ failure in the intensive care unit is well recognized.⁸²⁻⁸⁴ Many of these same processes are relevant to intra-operative care. Adequate resuscitation of hypovolemic shock may cause an ischaemia/reperfusion injury. This type of injury involves the previously described inflammatory pathways with activation of neutrophils and alterations in nitric oxide metabolism (reviewed by Lefer *et al.*,⁸⁵). Blocking neutrophil adhesion with a monoclonal antibody against CD18 has been shown to reduce organ injury in a primate model of haemorrhagic shock.⁸⁶ Major operative procedures such as abdominal aneurysm repair may also cause a reperfusion injury. Almost immediately following re-establishment of flow, there is loss of endothelial NO.⁸⁷ Later, there is an injury associated with increased neutrophil adhesion to endothelial surfaces. Part of this endothelial injury is mediated through a NO-dependent regulation of neutrophil adhesion^{88,89} and NO-dependent regulation of platelet-neutrophil interactions.²² It is likely that even the lung volume trauma associated with mechanical ventilation is

at least partially mediated through these same inflammatory pathways.^{90,91} This offers possibilities of new therapy based upon modulation of these inflammatory pathways. However, to date, large studies in septic shock have not shown improved outcomes following manipulation of these inflammatory pathways. Previous studies with glucocorticoids, potent inhibitors of iNOS induction,⁹² have not shown any positive effects in the treatment of established septic shock.⁸³ As well, a study utilizing a specific inhibitor of TNF α in human septic shock was discontinued prematurely because of increased mortality in the treatment group.⁹³ However, these treatments were aimed at established disease processes. It may be possible to give modulators of the inflammatory pathways in a prophylactic manner in the operating room setting, e.g. prior to reperfusion or prior to initiation of CPB.

III Summary

Although the pathways leading to nonspecific acute inflammatory injury are complex, an improved general understanding has emerged over the last decade. Following pro-inflammatory cytokine release there is activation of neutrophils and alterations in endothelial surfaces that promote neutrophil adhesion. At the same time there is increased generation of nitric oxide, an agent which confers organ protection under some conditions and under other conditions predisposes to organ injury. This may then result in a direct cellular injury and resultant organ impairment through the generation of peroxynitrite and toxic oxygen species. It is also clear that therapy aimed at blunting the inflammatory response may paradoxically worsen clinical outcomes.^{93,94} This likely represents a delicate balance that exists in which either under or over expression of the inflammatory response may be harmful (e.g. during CPB, either too much NO⁷⁷ or insufficient NO⁸⁵ may be harmful). This does not imply that modulation of parts of the pathways (e.g. adhesion molecules or of NO metabolism) cannot become clinically important tools in the future, but it does mean that, currently, these agents cannot be used outside selected controlled research settings. It is also likely that over the next decade modulation of these pathways will become common clinical practice and, therefore, an understanding of the interactions between mediators and effectors will become more important to the practising anaesthetist. Until definitive evidence becomes available, the balance of risk/benefit favours a "wait and see" approach.

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Glossary

Cardiopulmonary bypass	CPB
Coronary artery bypass grafting	CABG
Intercellular adhesion molecule	ICAM
Interferon γ	IFN- γ
Interleukin-1	IL-1
Interleukin-2	IL-2
Interleukin-4	IL-4
Interleukin-5	IL-5
Interleukin-6	IL-6
Interleukin-8	IL-8
Interleukin-10	IL-10
Multiple system organ failure	MSOF
Natural killer cells	NK cells
Nitric oxide	NO
Constitutive nitric oxide synthase	eNOS
Inducible nitric oxide synthase	iNOS
Peroxynitrite	ONOO ⁻
Platelet derived growth factor	PDGF
Transforming growth factor β	TGF- β
Tumour necrosis factor α	TNF α