

Innate Immunity in Surgical Patients

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Rezumat

Aspecte ale imunității înăscute la pacientul chirurgical

Sistemul imun înăscută reprezintă prima linie de apărare a organismului, ce prin mecanisme nespecifice este capabil să oprească agresiunea agenților patogeni. În acest articol sunt discutate aspecte relevante în înțelegerea acestor mecanisme, și anume modul de detectare a microorganismelor patogene de către sistemul imun, respectiv calea de semnalizare LPS-TLR precum și mecanismele de adaptare fiziopatologică și apărare a organismului împotriva activării excesive a sistemului imun înăscut, respectiv toleranța la endotoxină (ET). Toate aceste procese sunt controlate genetic la nivel translațional de o serie de fragmente de ARN non-codante (miRNAs). În final este discutată relevanța clinică în practica chirurgicală a imunității înăscute: reacția de fază acută postoperatorie, sepsisul, cazul particular al pacientului splenectomizat.

Cuvinte cheie: imunitate înăscută, receptori toll-like, toleranță la endotoxină, microRNA, reacție de fază acută, sepsis, splenectomie

Abstract

The innate immune system is the first line of defence against pathogens that acts immediately in order to prevent, control

and eliminate infections. This paper reviews some important aspects of innate immune sensing, namely the LPS-TLR signalling pathway and endotoxin tolerance (ET) as a host protective mechanism against uncontrolled immune activation. The fine-tuning of the innate immune response is enabled by miRNAs which constitute an additional level of gene expression regulation between messenger RNA (mRNA) and protein translation. Finally clinical relevance of this complex and dynamic process is pointed out: acute phase reaction, sepsis and the particular case of the splenectomised patient are discussed.

Key words: innate immunity, toll-like receptors, endotoxin tolerance, microRNA, acute phase reaction, sepsis, splenectomy

Introduction

The innate immune system also known as non-specific immune system is the first line of defence against pathogens. It is the primary, or early, barrier to infectious agents that acts immediately and serves two important functions: it is the initial response to microbes that prevents, controls and eliminates infections of the host and it can stimulate and influence the adaptive immune responses to make them optimally effective (1).

The elements of the innate immune system include anatomical barriers (i.e. the skin, internal epithelial layers) humoral and cellular components. When anatomical barriers are breached, another innate defence mechanism comes into play, namely acute inflammation. Part of the inflammatory response is the recruitment of polymorphonuclears, eosinophiles and macrophages to the sites of

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infection, which represent the cellular components of innate immunity and the main line of defence in the non-specific immune system through their capacity of phagocytosis. Furthermore, macrophages contribute to tissue repair and act as antigen-presenting cells, which are required for the induction of specific immune responses. Natural killer (NK) and lymphokine activated killer (LAK) cells – NK and LAK cells can non-specifically kill virus infected and tumour cells and are important in nonspecific immunity to viral infections and tumour surveillance. Eosinophils have proteins stored in granules that are effective in killing certain parasites (1,2).

Another important function of macrophages and NK cells is the secretion of cytokines that activate phagocytes and stimulate the cellular reaction of innate immunity. Because of that property cytokines are known to be intercellular mediators, so they play a central role in all interactions involving cells of the immune system. Cytokines that play a major role in the innate immune system include: TNF- α , IL-1, IL-10, IL-12, type I interferons (IFN- α and IFN- β), IFN- γ , and chemokines (3). There are proinflammatory and anti-inflammatory cytokines. Some proinflammatory cytokines examples are: IL-1, TNF- α , chemokines – which induce inflammatory reactions as response to microbes; IL-12 which is produced by macrophages and stimulates NK-cells to produce IFN- γ who acts on monocytes and macrophages to enhance cell mediated cytotoxicity (3). An example of anti-inflammatory cytokine is IL-10 which provides the control of inflammation by inhibiting the host immune responses, particularly responses involving macrophages (3).

Relevance of LPS signaling pathway for understanding innate immunity

The innate immune system senses pathogens largely through signals initiated by a collection of phylogenetically related proteins known as "Toll-like receptors" (TLRs). In humans, 10 TLR family members have been identified.

TLRs expressed on innate immune cells such as dendritic cells, macrophages and neutrophils detect molecular structures known as pathogen-associated molecular patterns (PAMPs) that are essential for the life-cycle of the pathogen (4). Effective sensing of PAMPs rapidly induces host immune responses via the activation of complex signalling pathways that culminate in the induction of inflammatory responses mediated by various cytokines and chemokines, which subsequently facilitate the eradication of the pathogen (5).

TLRs are type I membrane glycoproteins and consist of an extracellular leucine rich repeated domain (LRRs) that is required for PAMP recognition, and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain, required for downstream signalling.

The sensing role of the TLRs first came to light when one member of this family, TLR4, was shown to serve the detection of endotoxin (lipopolysaccharide; LPS) in mice. Each human TLR recognizes a distinct microbial molecule, or at most, a small collection of such molecules.

TLR signalling is primarily mediated via the recruitment of different TIR domain-containing adaptor molecules such as

MyD88, TRIF (TICAM-1), TIRAP (Mal), and TRAM to the TIR domains of the different TLRs. Recruitment of these adaptor molecules activates various transcription factors such as NF- κ B, IRF3/7, and MAP kinases to induce the production of pro-inflammatory cytokines and type I interferons in macrophages and cDCs (5-7).

For instance, in cDCs, UNC93B1 (a protein localized in the endoplasmic reticulum (ER)) plays a critical role in the transportation of endosome-localized TLRs. Mice with a mutation in this protein show complete abrogation of all cytokine production after stimulation with their respective PAMPs (8).

Studies, mostly performed on laboratory models may not reflect the true picture of host-pathogen interactions in the context of human disease (9), because pathogens consist of multiple ligands, which may activate multiple signalling pathways, and that this may lead to the crosstalk between various signalling pathways resulting in a wide range of innate immune responses. To date, human studies suggest that TLRs are not sufficient to mount protective immune responses against a wide range of infectious agents, suggesting that unidentified pathogen recognition receptors (PRRs) play a key role in defence against an array of pathogens.

WBS - a simple method to evaluate innate immunity

Innate tendency of immune activation is reflected in production of pro- and anti-inflammatory cytokines upon stimulation of Toll-like receptors (TLR) in whole-blood stimulation assays.

Whole blood stimulation (WBS) represents an excellent technique to study cytokine production, because it keeps the blood microenvironment and avoids the extraction procedure associated with modification of cell ratios and activation. It thus represents an ex vivo model of sepsis and the technique of choice to investigate inter-individual variations in cytokine production, because it mimics physiological conditions when compared to other techniques that would require cell isolation and extensive sample processing and manipulation (10).

By activating cells in whole blood samples with pathogen-associated molecular patterns (PAMPs), specifically with lipopolysaccharide (LPS), cytokine secretion can be conveniently evaluated. The whole blood assay requires minimal sample manipulation to assess cytokine production when compared to other methods that require labour intensive isolation and culturing of specific cell populations. It has to be noted that for this assay blood should be drawn into sodium heparin collection tubes as other anticoagulants are known calcium binders and can thus affect the results of the assay. Also crucial is the immediate processing of the samples once obtained.

ELISA (Enzyme-Linked Immuno Sorbent Assay) is a specific and highly sensitive method for quantitative measurements of cytokines. A specific monoclonal antibody (mAb) able to capture the cytokine of interest is coated on a microtiterplate. A second mAb, used for detection, binds a different epitope on the cytokine. The detection mAb is

labelled with biotin, which allows subsequent binding of a Streptavidin-conjugated enzyme. Any unbound reagents are washed away. When substrate is added, a colour reaction will develop that is proportional to the amount of cytokine bound. The concentration of cytokine is determined by comparison with a standard curve with known concentrations of cytokine.

miRNA regulation of innate immunity

Since their first observation in *Caenorhabditis elegans* in 1993, there is now overwhelming evidence that miRNAs are master regulators of gene expression in most cellular processes (11). They constitute an additional level of gene expression regulation between messenger RNA (mRNA) and protein translation.

The fine-tuning of the innate immune response by microRNAs (miRNAs) is a concept now supported by a rapidly growing body of evidence. Target prediction analyses indicate that up to a half of innate immune genes could be under the direct regulation of miRNAs (12).

An important aspect of miRNA regulation is the simultaneous regulation of several genes from the same functional network by one miRNA. In this respect, the field of innate immunity presents many unique opportunities. Signal transduction following pathogen-induced activation of immune sensors such as TLRs promotes the rapid induction of hundreds of genes that help in clearance of the pathogen (13). Only a few miRNAs (including miR-155, miR-146a, miR-21, miR-9) have been consistently found to be rapidly induced by innate immune activation (14,15). However, because these miRNAs are rapidly induced, it is reasonable to assume that they may play an important role in the modulation of the innate immune response.

miR-155 is one of the most studied miRNAs, which is induced by innate immune activation. It accumulates very rapidly following TLR and TNF- α stimulation. miR-155 expression is restricted to haematopoietic cells (B and T cells, macrophages, and dendritic cells) and it is overexpressed in cancers of B-cell origin. However, the basal level of miR-155 in immune cells is very low compared with other miRNAs such as miR-21 (14). In addition, blockage of miR-155 induction using synthetic antagomirs resulted in the derepression of genes involved in TLR/IL-1 signalling. Functionally, miR-155 has been implicated in the positive regulation of TNF- α production, the modulation of the IL-6 signalling pathway in B-cell lineage differentiation, and the inhibition of IFN- γ signalling in CD4 $^+$ T cells (14). EmmiR-155 transgenic mice produced more TNF- α when challenged with LPS. These functions of miR-155 on cytokine production and function point towards a combined action of complex gene networks. A recent study revealed that 100 proteins were significantly downregulated in HeLa cells, upon transfection of a miR-155 mimic. Among them, 28 have been involved in innate immunity which suggests that they could belong to similar gene networks, regulating TLR signalling and TNF- α production (15).

The discovery that miRNAs have altered expression patterns in cancer cells has created new opportunities in

cancer research and is directly related to the boom of the field of miRNA research (16). Not too surprisingly, miR-21, miR-155, and miR-146a, the prevalent innate immune miRNAs, have all been implicated in cancer and are considered as Onco-miRs (17). This further underlines their key role in the fine-tuning of innate immune signalling pathways. Additionally, translation of miRNA research into the clinic holds great potential, as recently demonstrated with HCV inhibition with miR-122 antisense treatment in chimpanzees (18). However, miRNA-regulation of gene expression should be seen as a novel opportunity to further our understanding of gene networks and uncover novel gene functions.

Endotoxin tolerance

While detection of pathogens by innate immune cells triggers a robust and essential inflammatory reaction, this process needs to be tightly regulated. Uncontrolled inflammation leads to extensive tissue damage and manifestation of pathological states like sepsis, autoimmune diseases, metabolic diseases and cancer (19).

Pathophysiological adaptations to regulate over-exuberant inflammation serve as important mechanisms for host protection against endotoxin shock (20). Endotoxin tolerance (ET) is the classic example of such a protective mechanism, a phenomenon in which cells or organisms exposed to low concentrations of endotoxin (e.g. LPS) enter into a transient unresponsive state and are unable to respond to further challenges with endotoxin; in other words, they develop a kind of "tolerance" to endotoxin (21). Clinically, this state is associated with monocytes/macrophages in sepsis patients where they contribute to "immunosuppression" and mortality. Some investigators suggest that the host response to sepsis consists of concurrent processes involving both exaggerated inflammatory and immunosuppressive states. However, the failure of anti-inflammatory therapies to effectively target human sepsis and the fact that mortality due to sepsis often occurs in immunocompromised patients, advocates a crucial role for the immunosuppressive phase in sepsis (22). Blood monocytes from sepsis patients show several characteristics similar to ET. This is evident by their drastically decreased production of proinflammatory cytokines like TNF α , IL-6, IL-1 α , IL-1 β and IL-12, upon ex vivo LPS challenge as compared to that of monocytes from healthy individuals. At the same time, upregulated expression of anti-inflammatory cytokines like IL-10, TGF β and IL-1RA was reported in these cells (23).

The molecular basis of how monocytes/macrophages "switch" from an inflammatory to an immunosuppressive phenotype in sepsis remains elusive. It is believed that excessive inflammatory signals in the early phase of sepsis induce these cells to adopt an endotoxin-tolerant state. It has been proposed that signals that promote inflammation ("GO" signals) can also lead to signals that tune-down inflammation ("STOP" signals). For example, COX2, which is highly expressed by sepsis macrophages, is responsible for prostaglandin E2 (PGE2) production. Accumulating levels of PGE2 can inhibit COX2

expression in a negative feedback manner and stimulate the production of anti-inflammatory compounds like lipoxins (24).

In vitro studies of endotoxin-tolerant murine macrophages and human monocytes, along with their recent transcriptomal characterization showed that most of the genes downregulated upon LPS re-stimulation were inflammatory cytokines and chemokines like TNF α , IL-6, IL-12, IL-1 β , CCL3, CCL4 and CXCL10 (25). Upregulated genes were more varied, consisting of anti-inflammatory cytokines such as IL-10, TGF β and IL-1RA; scavenging C-type lectin receptors such as MARCO, CLEC4a and CD64; negative regulators such as IRAK-M and a variety of anti-microbial genes (e.g. FPR1, AOA and RNASET2). The upregulation of these genes in tolerant cells points to a profound “gene reprogramming” rather than an overall downregulation of LPS-induced gene expression (26).

Defects in TLR4 signalling have been observed at the level of the receptor, adaptors, signalling molecules, and transcription factors. In vitro, ET in mouse macrophages and human monocytes has been associated with decreased TLR4–MyD88 complex formation, impairment of IRAK-1 activity, defects in the activation of mitogen-activated protein kinases (MAPKs) and NF- κ B (27).

MicroRNAs, which have an important post-transcriptional regulatory role for gene expression also have influence on ET. This arises from the finding that proinflammatory stimuli like LPS, TNF α , and IL-1 β induce the expression of specific microRNAs that, in turn, affect the TLR4 and IL-1 receptor (IL-1R) signalling pathways in monocytes/macrophages (28). David Baltimore's group initially demonstrated that LPS treatment augments the expression of two microRNAs, miR146 and miR155, in human monocytic cells. miR146 downregulates inflammation by attenuating IL-1R or TLR4 signalling through post-transcriptional regulation of IRAK-1 and TRAF6 proteins (29). In fact, dramatic downregulation of IRAK-1 protein but not its mRNA during ET has been observed, which fits with the possibility of a microRNA-mediated regulation. miR146 is induced by a variety of inflammatory stimuli, including IL-1 β and TNF α , and ligands for TLR2 and TLR5 (30).

miR155 has been implicated in LPS response and endotoxin shock models. LPS and double-stranded RNA induce miR155 through autocrine and/or paracrine induction of TNF α via type I Interferon. Because both TNF α and IFN β are known mediators in LPS shock, the upregulation of miR155 is conceivable during sepsis or ET. miR155 attenuates TLR4 signalling by targeting IKK ϵ directly. However, at the translational level, miR155 was shown to promote TNF α translation (15).

Another microRNA, miR125b, has been found to target the 3'-untranslated region of the TNF α transcript, thereby inducing its degradation. This report suggests an oscillatory behaviour of miR155 versus miR125b in the regulation of TNF α expression during LPS stimulation: miR155 is upregulated, while miR125b is downregulated (31).

Finally, differential regulation of several cytokines and chemokines in LPS-treated or LPS-tolerant cells has been attributed to differential mRNA and protein stability, suggesting a more fundamental role for microRNAs in regulating

inflammatory cytokines and chemokines. Perhaps the actual role of microRNAs in the control of ET could be at the late phase of inflammation rather than the early events (23). This can be extrapolated from the fact that microRNAs such as miR155, miR146a and miR9, which are upregulated by LPS or endotoxin shock, are likely to act as a negative feedback loop in the later phase of inflammation, inhibiting the TLR4 pathway and thus promoting ET (23). However, these microRNAs may act at multiple levels, such as perturbing TLR signalling molecules, regulating the stability of downstream cytokines or even positively regulating some negative regulators.

In conclusion, the progress made in our understanding of inflammation, TLR signalling and gene expression profiling of monocytes/macrophages has called for a review of ET in the light of these findings. Several salient points have emerged.

First, ET can be viewed as a negative feedback response arising as a result of dysregulated inflammation (e.g. sepsis) (23,32).

Second, ET is a case of gene reprogramming and immunomodulation rather than a global downregulation of gene expression and function. In this context, the use of the term ‘tolerance’ could be misleading (23).

Third, the regulation of ET is multi-levelled, involving receptors, signalling molecules, negative regulators and post-transcriptional changes like chromatin remodelling and microRNA regulation (23,33,34).

Finally, an endotoxin-tolerant state is restricted to sepsis or SIRS, and is observable in many diseases, such as hepatic ischaemia, acute coronary syndrome, cystic fibrosis and perhaps even cancer. Thus, lessons learnt from ET might be relevant to the understanding of other diseases. However, whether ET represents a general paradigm for immunosuppression across different disease states has yet to be determined.

Clinical relevance of innate immunity: acute phase reaction

The acute phase response (APR) is an innate body defence seen during infection, tissue injury, trauma or surgery, neoplasia or immunological disorders and involves the increased production of certain blood proteins termed acute phase proteins (APP). Each form of injury or tissue disorder that precipitates an inflammatory response inevitably also causes an acute phase reaction, which is a biologic period of repair when the host restores homeostasis (35). However, excessive APR may be harmful leading to host self-damage (36).

At the site of injury pro-inflammatory cytokines are released, and the vascular system and inflammatory cells are activated. Activated macrophages and other leukocytes release inflammatory cytokines among which the most important are TNF-alpha, IL-1 and IL-6 (37). The initial reaction is the release of IL-1 and TNF- α from activated macrophages and monocytes in the damaged tissues. This stimulates the production and release of IL-6, the main cytokine responsible for inducing the systemic changes that occur during APR: fever, tachycardia, acceleration of catabolism (38). These responses in turn are associated with production of more cytokines and other inflammatory mediators

which diffuse to the extracellular fluid compartment and circulate in the blood. The cytokines travel through the blood and stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins (i.e. C-reactive protein -CRP) associated with a decrease in synthesis of normal blood proteins (i.e. albumin) (39), due to hepatic mRNA upregulation of those APPs (40).

The acute phase response with its changes in blood plasma composition is beneficial to the organism by preventing microbial growth and helping to restore homeostasis. Some APPs opsonize microorganisms and activate the complement system, others remove cellular remnants and free radicals, or neutralize proteolytic enzymes.

At present, it is well established that the magnitude of this response is proportional to the severity of trauma (41) and plasma levels of APP allows quantification of APR to the surgical trauma (42). The response is initiated locally, at the site of injury (43) (i.e. surgical wound) where activation of immune cells stimulate the release of cytokines. With respects to this many studies demonstrated that the minimally invasive approach produces lesser surgical trauma and allows for a lower intensity of APR. This advantage is even more important in patients undergoing major surgery for malignancies with increased degree of intraabdominal lesion, operative time, blood loss, intestinal manipulation and anastomoses. In these cases, besides the cutaneous stimulus all the factors enounced contribute to innate immune activation. Minimally invasive approach involves an attenuated postoperative response related to smaller cutaneous incision, intraoperative blood loss and intestinal manipulation and, in consequence, a more rapid recovery after surgery. Moreover, reduced APR and preservation of postoperative immune response in patients with neoplasms and thus with an immune system which is previously depressed by the disease itself, favour not only postoperative recovery, but also may influence the evolutive course of the disease (44).

SEPSISurgical

In the past, sepsis was commonly thought to be caused by overactivation of the innate immune system, and the ensuing pro-inflammatory cascade, in response to severe microbial infection or extensive tissue damage (45). However, the failure of anti-inflammatory therapies for sepsis in clinical trials raised the question of whether mortality in sepsis actually derives from an uncontrolled pro-inflammatory response (46). Although some patients die during the initial, hyperinflammatory phase of sepsis, most patients succumb at later time points that are associated with a prolonged immunosuppressive state (Fig. 1).

During sepsis, homeostasis between the various biological systems of the inflammatory network is highly imbalanced. In the initiation of sepsis, the release of a large amount of PAMPs results in the overstimulation of pattern-recognition receptors (PRRs) on immune cells. Activated immune cells release excessive amounts of pro-inflammatory mediators (resulting in a 'cytokine storm'), free radicals and enzymes, which convert the normally beneficial effects of inflammation into an excessive response that produces host damage and cell

apoptosis. Apoptosis of lymphocytes and dendritic cells (DCs) further contribute to the suppression of immune responses during sepsis (47). In addition to causing a marked decrease in cell numbers, the apoptosis of lymphocytes and DCs contributes to immunoparalysis through the immunosuppressive effects of apoptotic cells. In contrast to lymphocytes and DCs, the apoptosis of macrophages and neutrophils seems to be unaffected or even decreased during sepsis. Whereas the increased apoptosis of lymphocytes and DCs results in severe immunosuppression, which places the patient at risk of nosocomial infections, decreased neutrophil apoptosis increases the damage caused by their pro-inflammatory activity (48).

In conclusion, none of the therapeutic approaches for sepsis that target the inflammatory response has decreased rates of sepsis mortality. In addition, it is now clear that sepsis is a complex, dynamic syndrome with great heterogeneity, and not a distinct disease. Sepsis can result from various causative insults, and susceptibility can be influenced by numerous factors. In particular, genetic and epigenetic changes, such as mutations in genes that encode PRRs or mediators of inflammation and their receptors, might have consequences for the host response (49,50). Due to their important role in repression of pro-inflammatory cytokines translation, microRNAs have a significant influence in sepsis pathogenesis (51). In sepsis patients abnormal levels of the several types of microRNA were found: miR-146, miR-155, miR150, miR-132 (50,52).

Ideally, individual patients should be precisely monitored for changes in characteristic markers of the host immune

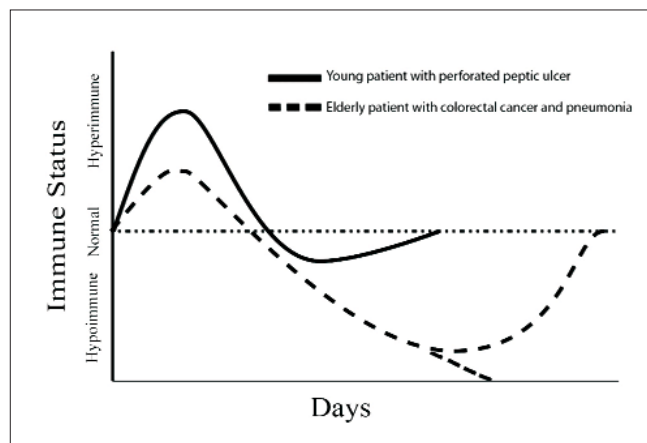


Figure 1. Immune response of two hypothetical patients with sepsis. The individual response is determined by many factors, including the virulence and size of the inoculum, and the patient's coexisting conditions, age, and polymorphisms in genes for cytokines. In the hypothetical young patient who has contracted a serious peritoneal infection, there is an initial robust hyperinflammatory response, but if infection resolves rapidly, there is only a minimal hypoimmune state. In the hypothetical elderly person with colorectal cancer, the initial response is limited, and, if infection persists, a prolonged hypo-inflammatory response develops, followed by either recovery or death.

response to aid in the choice of specific immunomodulatory therapies.

Particular case of splenectomy

The spleen is a lymphatic organ that plays a fundamental role in protecting the body from invading pathogens. The white pulp, which represents the immunological compartment of the spleen, is divided into B and T lymphocyte zones. The spleen combines the innate and adaptive immune system in a unique way, releasing an immediate innate reaction to microbial penetration, but also an adaptive immune response that involves the interaction of cells that recognize a particular antigen, implicating Major Histocompatibility Complex (MHC) molecules presented by antigen-presenting cells (APC) (53). This means that the spleen plays a fundamental role in bacterial clearance either by antibody response or macrophage bactericidal capacity. At the same time, there is evidence that the spleen also contributes to bacterial endotoxin detoxification (54). PALS (periarterial lymphatic sheaths) are the T lymphocyte zone of the spleen, while B cell-lineage lymphoid follicles (primary or secondary) appear as outgrowths of the PALS (53). Another important area is the marginal zone which surrounds the primary follicle or mantle zone of the secondary follicle. The marginal zone seems to be a primary site of entry of B and T cells where they meet antigens derived from the circulation. In the marginal zone, memory B cells can be found following a primary immune response. As far as splenic macrophages are concerned, as innate immune effectors, those contained in the red pulp are less differentiated, express high levels of MHC II molecules and engulf polysaccharides from the capsule of *S. pneumoniae* (55). Macrophages in the marginal zone are more differentiated with limited expression of class II MHC molecules and are in intimate contact with B cells (55).

The phagocytic capacity of splenic macrophages is fundamental for clearing bacteria from the blood, which is less efficient after splenectomy. Marginal zone macrophages in the spleen act as APCs toward bacterial polysaccharides, inducing IgM-specific anti-polysaccharide antibody-containing cells in the spleen (56).

The capacity of the spleen to modulate the alveolar macrophage bactericidal activity has been investigated since splenectomy in mice impairs alveolar macrophage function. In particular, the role of IL-1 and Granulocyte-Colony Stimulating Factor (G-CSF) has been analysed. The activity of these cytokines was quite different depending whether the mice were splenectomised or not, suggesting that the spleen may generate factors that enhance alveolar macrophage function (57). In addition, evidence has been provided that G-CSF can protect the host against the lethal effects of LPS by suppressing tumour necrosis factor (TNF)- α release.

However, the spleen plays a specific role in the defence against encapsulated bacteria. This is mainly related to the marginal zone containing marginal zone B cells (MZ B cells) and macrophages. MZ macrophages are able to capture whole encapsulated bacteria from the circulation and initiate a

humoral immune response. MZ B cells are a distinct B cell lineage that develop and mutate immunoglobulin (Ig) receptors during the first years of life without being engaged in any immune response (58). Upon stimulation with antigens expressed by encapsulated bacteria, the prediversified MZ B cells can rapidly proliferate and differentiate into APCs or into IgM-, IgG-, and IgA-secreting plasma cells, circulating for several months. MZ B cells do not differentiate into memory cells and are therefore part of the (immediate) innate immunity against invading pathogens (58).

After splenectomy, the mechanisms involved in bacterial clearance are altered, leading to gram-positive, but also gram-negative sepsis, known as OPSI (overwhelming post-splenectomy infection), which has a fulminant course and mortality ranges from 50% to 70% (59). OPSI is caused mainly by encapsulated gram-positive bacteria such as *Streptococcus pneumoniae* (which is the aetiological agent in about 80% of cases), *Neisseria meningitidis* and *Haemophilus influenzae* which induce sepsis. With special reference to the pathogenetic mechanisms, it seems that gram-positive sepsis may share some similarities with gram-negative sepsis, mostly in terms of release of noxious mediators and cytokines. Previously the ability of cell walls from gram-positive bacteria to interact with signal-transducing molecules such as CD14 was reported. However, other investigators have demonstrated that group B streptococci type III (GBS) activated TNF production from human monocytes in a CD14-independent fashion (60). Gram-negative sepsis is caused by LPS released from bacterial cell wall into host tissues which binds to the CD14 receptor and TLR on macrophages, thus triggering the release of proinflammatory cytokines, such as IL-1b, IL-6, TNF α with the ensuing inflammatory cascade.

Conclusively, the spleen has an important role in the host protection against bacterial pathogens and its removal should represent a difficult medical decision and not an indiscriminate surgical manoeuvre (61,62). For instance, in patients with high risk for sepsis, such as hepatitis C virus infected subjects, patients with inflammatory bowel disease and gastrointestinal cancer patients splenectomy is not advisable in order to avoid fatal complications (54).

Conclusion

Pathogens threaten us continuously but we are equipped with powerful defence mechanisms. The first line of defence, innate immunity, can destroy invading microorganisms and trigger inflammation that contributes to blocking their assault. There are multiple pathways for inducing the spectrum of mediators required to mount an effective innate immune response. This involves multiple receptors and downstream pathways that integrate extracellular cues. When these pathways act in concert, host inflammatory responses are reduced. In other settings, there are dysfunctional responses that lead to human disease.

The components of the immune system have been identified step by step during the 20th century, but there still are lessons to be learned from the myriad ongoing studies that may

lead to new treatment options for maladies as sepsis and cancer which represent major challenges for scientists and clinicians and are a tremendous burden for health-care systems.

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