1. Introduction

Sepsis strikes over 750'000 patients in the United States annually, of which more than 210'000 do not survive. Surprisingly, its incidence continues to increase with an estimated rate of ~1,5% per year (1, 2). In 1989, the annual incidence of sepsis was 175 cases in 100'000 people, and this incidence has been steadily increasing with current levels of more than three-fold of those seen in the early 1970s (3, 4). Despite continuing progress in the development of antibiotics and other supportive care therapies, sepsis remains a leading cause of high morbidity and mortality in intensive care units (5). Septic shock and sequential multi-organ failure or dysfunction syndrome (MOFS/MODS) correlate with poor outcome (6). Yet, outcome from sepsis syndrome and shock has not improved significantly in the past 50 years, representing the 12th most common cause of death in the United States (1, 2, 4). Furthermore, results from the administration of anti-inflammatory agents and anti-cytokine therapies in clinical trails have, in general, been disappointing (7-10).

1.1. Definition of sepsis

Sepsis has been historically defined as a clinical syndrome comprised of fever, leukocytosis (or leukopenia), elevated cardiac output, and reduced systemic vascular resistance associated with a severe infection (11, 12). However, the term "sepsis" has been recently supplanted with the term "sepsis syndrome" in deference to patients manifesting the physiologic and metabolic responses associated with sepsis, but without a documented severe infection. It is important to realize that the terms "sepsis" and "sepsis syndrome" refer exclusively to the *physiological* and *metabolic responses* to severe infection and/or tissue injury. They do not commonly refer to the *immunological status* of the patient. There has been an effort in recent years to describe the septic patient more precisely in terms of their immunological status. For example, the term "Systemic Inflammatory Response Syndrome (SIRS)", is now frequently associated with the nonspecific, systemic activation of the innate immune system and human proinflammatory cascade seen during sepsis syndromes. In contrast, the term "Compensatory Anti-inflammatory Response Syndrome (CARS)" has been used to define immunologically, those patients with sepsis syndromes who

are manifesting predominantly a pattern of macrophage deactivation, reduced antigen-presentation, T-cell anergy and a shift in the T-helper cell pattern to a predominantly Th_2 type (humoral immunity) response. Patients manifesting a more mixed pattern of response have been designated as having a "Mixed Anti-inflammatory Response Syndrome (MARS)" (13-15). Although the definitions are by no means established among investigators, nor are they used consistently, they are helpful in at least focusing on the immunological status of patients with sepsis syndromes.

1.2. Activation of the innate immune system

The principal components of the innate immunity are either physical and chemical barriers, such as epithelial and antimicrobial substances produced at epithelial surfaces, or blood proteins including members of the complement system and other mediators of inflammation (cytokines), as well as neutrophils, macrophages, and natural killer cells (phagocytic-mediated immune response). Innate immunity provides
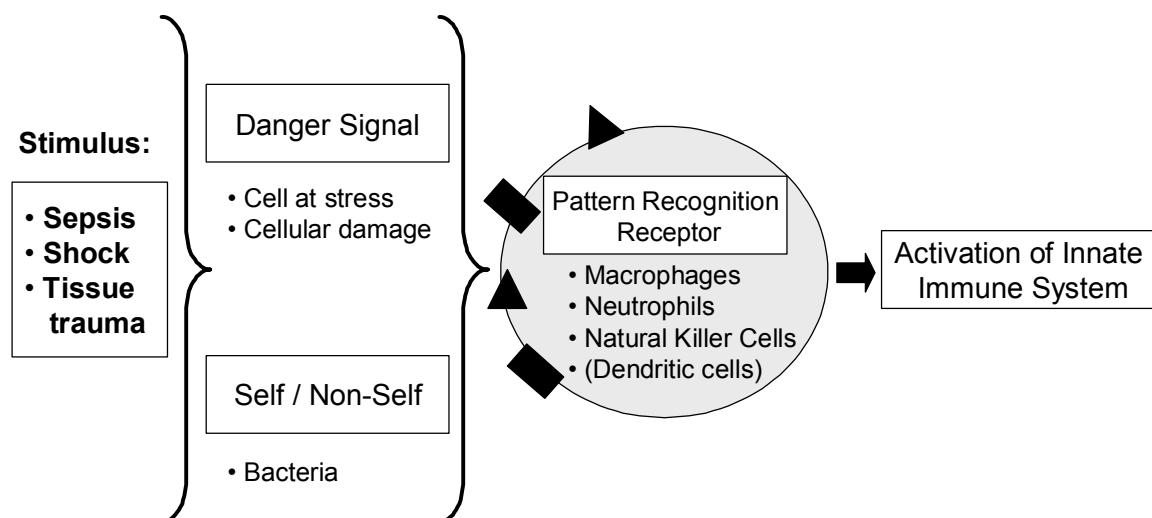
Stimulus:

- **Sepsis**
- **Shock**
- **Tissue trauma**

Danger Signal
- Cell at stress
- Cellular damage

Self / Non-Self
- Bacteria

Pattern Recognition Receptor
- Macrophages
- Neutrophils
- Natural Killer Cells
- (Dendritic cells)

Activation of Innate Immune System

Fig. 1: Two different ways to activate the innate immune system. The innate immune systems relies on cell surface receptors, called pattern recognition receptors, which can either recognize invading microorganisms (self/non-self) or endogenous signals of distressed or damaged cells (danger signals).

the early nonspecific host defense against microbes as well as debris and necrotic/apoptotic cells after tissue injury (16). Presently, it is recognized that there are two principal mechanisms by which the innate immune system can be activated: either by endogenous signals of distress or cell damage (self antigen) or by the presence of invading microorganisms or their products (nonself antigen) **(Fig. 1)**.

The proposition, initially formed by Charles Janeway, stated that the innate immune system relies predominantly on the discrimination between self and nonself entities associated with pathogens has been generally accepted for nearly a decade (17). More recently, Polly Matzinger proposed, that the innate immune system is less concerned with the differences between self and nonself as it is about to protect itself against danger (18). Based on this latter proposal, which has been recently termed the "danger model" (18, 19), host innate immunity has also evolved to recognize endogenous signals of distress (heat shock proteins, nitrosylated proteins, DNA adducts) or cellular damage (necrotic cells/tissues), and therefore does not require foreign or infectious agents for initiation. This latter model may explain the activation of innate immunity and a systemic inflammatory response to nonmicrobial challenges after severe tissue injury including mechanical trauma, ischemia/reperfusion injury, and hemorrhagic shock. The early induced but non-adaptive responses to tissue injury and invading microorganisms are based on non-clonally distributed receptors called pattern recognition receptors that recognize certain molecular patterns, also termed pathogen-associated molecular patterns (14, 20-22) **(Fig. 1)**.

1.3. Role of cytokines during inflammation

A vigorous induction of the innate immune system can and often does have catastrophic effects on patients with severe trauma or sepsis. Exaggerated production of proinflammatory cytokines and the induction of more distal mediators such as nitric oxide, platelet activation factor, and prostaglandins have been implicated in the endothelial changes and induction of a procoagulant state that leads to hypotension, inadequate organ perfusion, and necrotic cell death associated with "Multiple Organ Dysfunction Syndrome" (MODS) (23). This proinflammatory state has been defined as "Systemic Inflammatory Response Syndrome" (SIRS) **(Fig. 2)** (13). However, a large majority of these patients survive this initial SIRS event, and the
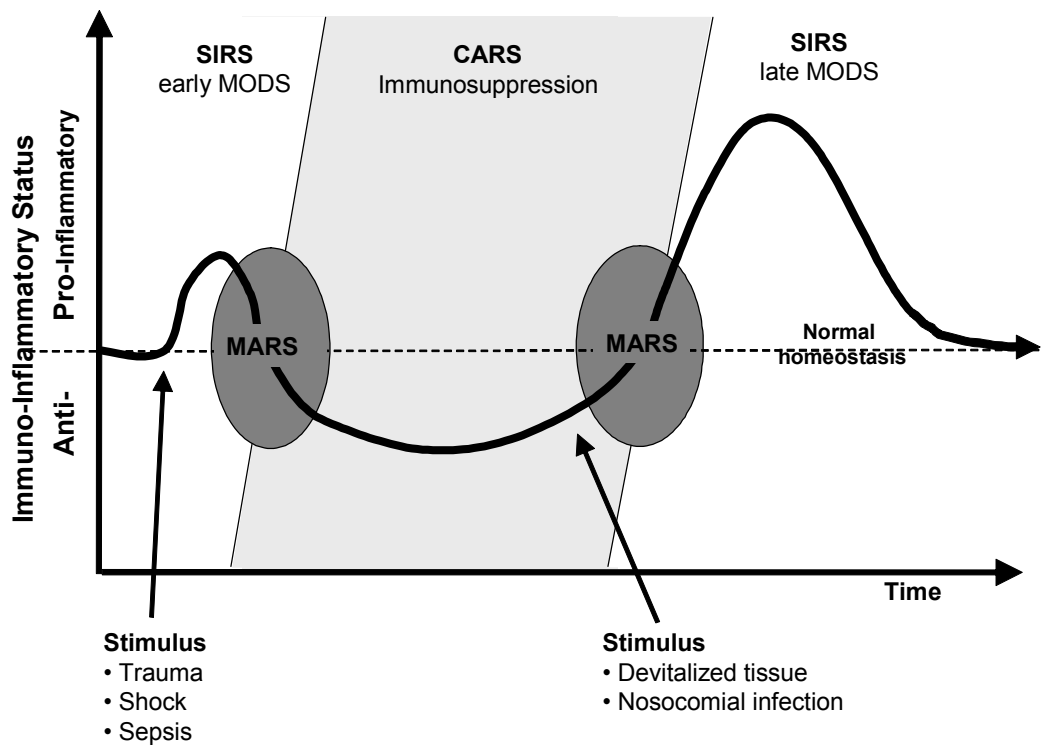
Fig. 2: Time course of an immunological profile after severe trauma or sepsis. The multimodal nature of soft tissue injury and sepsis response is often characterized by a brief proinflammatory (SIRS) period and a sustained immunosuppressive or anti-inflammatory profile (CARS) leading to increased susceptibility for nosocomial infection, and therefore the risk for a second proinflammatory insult.

proinflammatory state ultimately resolves. Paradoxically, the proinflammatory cytokines and humoral mediators responsible for the induction of the innate immune response and SIRS also contribute to the development of acquired or specific immune defects. The patient often enters an immunological state characterized by T-cell hyporesponsiveness, anergy, and a defect in antigen presentation called "Compensatory Anti-inflammatory Response Syndrome" (CARS) **(Fig. 2)** (15). CARS is characterized by defects in antigen presentation, macrophage "paralysis", T-cell anergy, suppressed T-cell proliferation, decreased Th_1 cell proliferation, and an increase in T-cell and B-cell apoptosis (24). Patients with severe trauma or sepsis are often anergic and show an increased susceptibility to invading microorganisms through inhibition of the cellular, humoral, and phagocytic immune system (25). Typically, after severe tissue trauma or sepsis an early proinflammatory response is

followed by a more sustained anti-inflammatory response. However, the temporal relationship between a proinflammatory and an anti-inflammatory cytokine response has not been fully delineated, and patients may move temporally and repeatedly between proinflammatory and anti-inflammatory states. Patients manifesting a more mixed pattern of response have been designated as having a "Mixed Anti-Inflammatory Response Syndrome" (MARS) **(Fig. 2)**. Although the timing for the development of a SIRS or CARS response will vary, there is general consensus that the interaction between the proinflammatory and anti-inflammatory mediators ultimately determines the severity of immune dysfunctions and the outcome of the patient. Finally, either a balance is achieved and homeostasis is restored or the proinflammatory (TNF-α, IL-1, IL-6, IL-18) and anti-inflammatory (IL-4, IL-10) mediators, respectively, take the lead causing SIRS or CARS and, ultimately result in MODS and "Multiple Organ Failure" (MOF) **(Fig. 2)** (14).

1.4. Role of apoptosis during inflammation

There has been growing recognition that the response to human sepsis or systemic inflammatory response syndromes is not always dominated by a proinflammatory state, but is frequently associated with immune suppression and an increased loss of lymphocyte populations from the thymus, spleen, bone marrow, and intestinal tissues. Early preclinical studies with anti-TNFα and anti-IL-1 therapies relied primarily on rodent and primate models of endotoxemia, or Gram negative bacteremia, which focused predominantly on the exaggerated proinflammatory response rather than the associated immune suppression (26, 27). In these models, inhibitors of inflammation often improved outcome (28, 29). However, these same therapies were much less effective in rodent models of sepsis which arose from a nidus of infection, like the cecal ligation and puncture model, or from a thermal injury, and were commonly associated with immune suppression (30, 31). Unlike endotoxemia or Gram negative bacteremia, but similar to human sepsis, these models were not generally associated with high circulating concentrations of the proinflammatory cytokines, TNFα and IL-1. Rather, these rodent studies in particular were noteworthy because of an apoptotic loss of CD4+ T- and B-lymphocytes which occurred rapidly (usually within hours) after the induction of sepsis. Increased apoptosis in lymphoid tissues of the spleen, thymus

and small intestine were all observed within three hours after a nonlethal scald burn (32). Similar findings were observed within 24 hours after generalized peritonitis (33, 34) **(Fig. 3)**. The apoptotic cascade initiated by cecal ligation and puncture in rodent models of sepsis led to increased caspase-9 and caspase-3 activity in the thymus
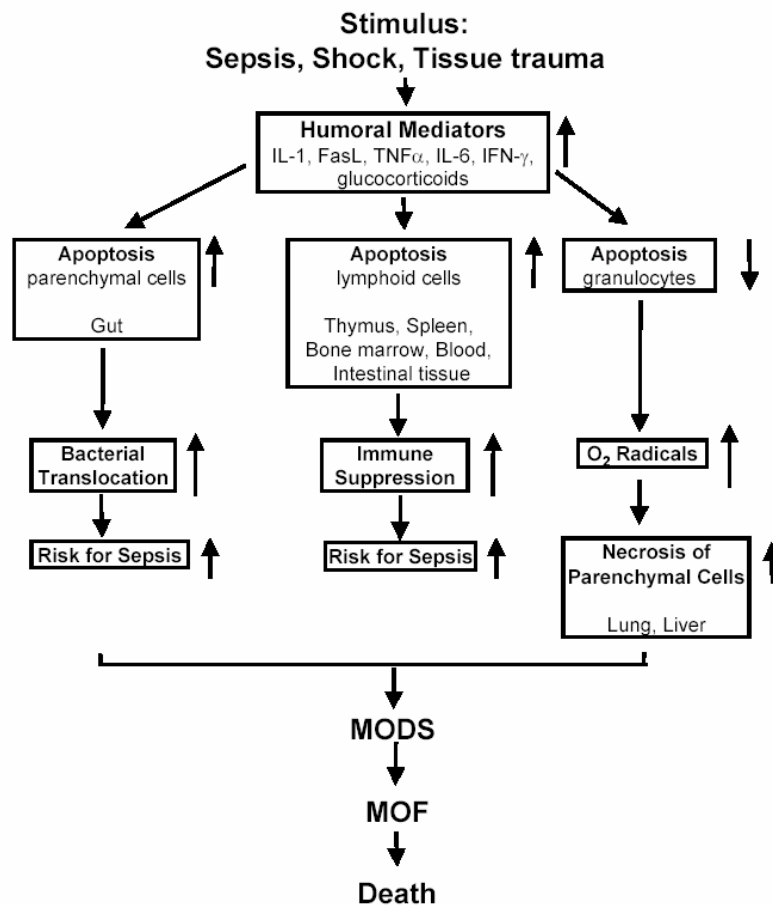

Fig. 3: Apoptosis dependent pathways for the development of sepsis induced multisystem organ dysfunction syndrome (MODS) and/or multi organ failure (MOF)

(35-37). Increased caspase-3 and -9 activity appeared to play critical roles in apoptosis since administration of a caspase-3 inhibitor reduced thymocyte apoptosis in mice after a cecal ligation and puncture (35) and a thermal injury (32). In addition, thymocytes deficient of caspase-9 were resistant to glucocorticoid induced apoptosis (38). Furthermore, tissue expression of humoral factors including tumor necrosis

factor α (TNFα) and Fas ligand (FasL), both known to induce apoptosis in lymphoid tissues, were modestly increased in these experimental models (34, 39-41). Similarly, glucocorticoid concentrations, an additional inducer of T-cell apoptosis, were also frequently increased (42). On the other hand, decreased apoptosis of neutrophils, which is often seen during inflammation, has been shown to correlate with deficiency of the proapoptotic molecule Bax (43).

Only recently, however, have these findings originally reported in rodents been confirmed in human sepsis. A recent clinical report suggested that more than 50% of patients who died from sepsis exhibited depleted splenic white pulp and increased lymphocyte apoptosis in this organ (36). These findings raised the intriguing possibility that a large proportion of critically-ill septic patients may also be undergoing accelerated apoptotic processes which resulted in the depletion of lymphoid populations. In the same report, approximately 80% of the patients who died from sepsis syndromes and their accompanying multisystem organ dysfunction (MODS) were lymphopenic. Other investigators have also shown a correlation between lymphopenia in trauma patients with outcome and development of sepsis and multiorgan failure (MOF) (44, 45). The lowest counts of lymphocyte subsets were noted in patients who developed infection or died. However, lymphopenia does not always correlate with organ lymphocyte depletion (36), in part because blood lymphocyte numbers are regulated by several factors including lymphocyte trafficking, apoptosis and adherence to the vascular endothelium and diapedesis.

Surprisingly, however, this increased apoptosis is not as frequently seen in parenchymal tissues **(Fig. 3)**. Rodent studies in particular have demonstrated that increased apoptosis is primarily limited to tissues and organs rich in lymphoid cells, like the bone marrow, spleen and thymus, and to a lesser extent the gut (32, 46). Furthermore, increased apoptosis appears to be primarily restricted to CD4+ helper T-cells, some immature T-cell populations, and B-cells in these tissues. These findings have been confirmed in humans, at least in a preliminary fashion. In the liver, kidney and lungs of patients with sepsis-associated organ failure, apoptosis of parenchymal or epithelial cells does not appear to be present (36).
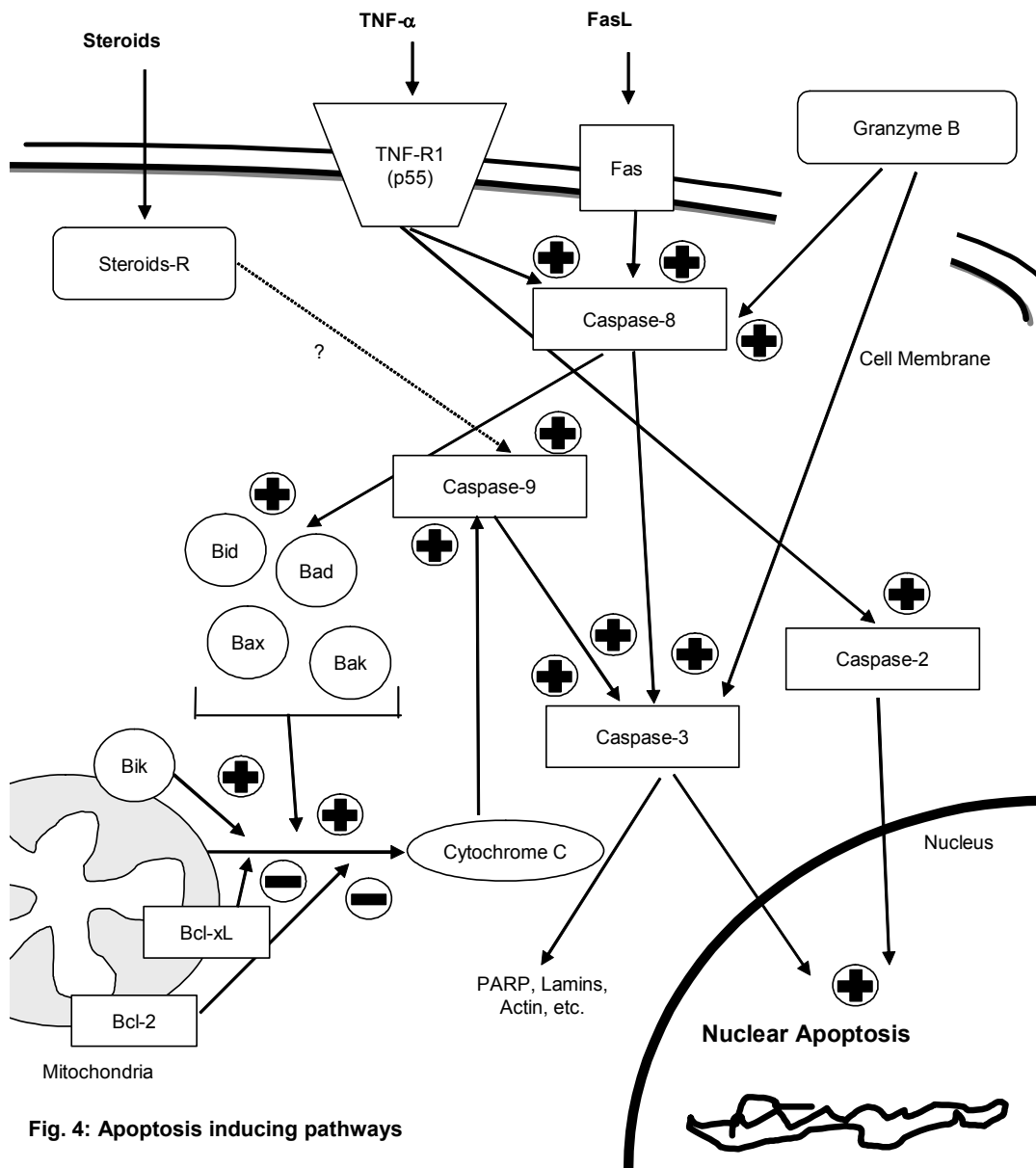
Fig. 4: Apoptosis inducing pathways

Immune cells play a key role during inflammation. Inhibition of their functions, dysbalanced ratios of cell numbers or chronic activation represent the hallmarks of inflammation. While lymphocyte and monocyte/macrophage functions (25, 47, 48) are often depressed during systemic inflammation, neutrophils reveal a chronic stimulation with an excessive tissue load at sites of inflammation. Depression of lymphocyte and macrophage functions with interruption of connecting pathways (monocyte/macrophage-Th$_1$-cellpathway) cause a generalized suppression of the cellular immune response with increased susceptibility to penetrating microorganisms

(12, 25, 47, 49). In contrast, neutrophils which are the first cells to migrate to the scene of tissue perturbation (50, 51), release a large number of toxic metabolites (proteases, O_2^--radicals) (50, 52, 53), but also cleave matrix proteins into chemotactic factors (54) with the potential to amplify inflammation by attracting more cells.

It can be hypothesized from these studies that apoptosis represents a key mechanism in the control of immune cells during inflammation. This is supported by the fact that most of the cells of the hematopoietic system have short life expectancies, from less than a day for blood neutrophils to a few weeks for monocytes and lymphocytes (55). Because uncontrolled immune responses are potentially dangerous alterations for the host, they must be carefully regulated or extinguished, if the antigenic stimulus exceeds a tolerable threshold. Therefore, one important mechanism of immune cell homeostasis is "programmed cell death" (apoptosis). The process of apoptosis can be subdivided into three different phases: initiation, effector stage, and degradation (56-59). Whereas the initiation stage depends on the type of apoptosis-inducing stimulus, the effector (which is still subject to regulation) and degradation (beyond regulation) stages are common to all apoptotic processes and are irreversible. The programmed cell death is usually regulated by the cellular environment. Another cell may make the decision whether a particular cell lives or dies, either by secreting or expressing a "death factor" (e.g. Fas ligand) on the cellular surface (58, 60-63), or by depriving the cell of an essential "survival factor" (e.g. interleukin-2 for lymphocytes) (64, 65).

Studies in experimental animals and critically ill patients have demonstrated that increased apoptosis of lymphoid organs and parenchymal tissues contributes to suppression of the immune system, anergy, and organ system dysfunction (24, 55, 66, 67). During systemic inflammation, lymphocyte apoptosis can be triggered by the absence of IL-2 or by the release of glucocorticoids, granzymes, or the so-called "death" cytokines, tumor necrosis factor-α and Fas Ligand **(Fig. 4)**. Apoptosis proceeds via auto-activation of cytosolic and/or mitochondrial caspases, which can be influenced by the pro- and anti-apoptotic members of the Bcl-2 family. Direct apoptotic organ injury and the immune suppression secondary to apoptotic losses in T-cell, B-cell, and NK cell populations may contribute significantly to the risk of secondary opportunistic sepsis and finally MODS. While lymphoid cells are

undergoing accelerated apoptosis, spontaneous neutrophil apoptosis associated with systemic inflammation is delayed (68-70).

Recent studies in experimental animals and critically ill patients have suggested that increased apoptosis of lymphoid organs and some parenchymal tissues may contribute to the immune suppression, anergy and organ system dysfunction. Although sepsis is associated with multiple derangements in host physiology and immunology, this increased lymphoid cell apoptosis appears to contribute to adverse mortality. Animal studies have demonstrated that blocking apoptosis can improve outcome in experimental models of severe sepsis. Caspase-3 represents a primary effector in the apoptotic cascade and administration of caspase-3 inhibitors prevents mortality in a murine model of generalized peritonitis (35). Moreover, overexpression of the anti-apoptotic Bcl-2 protein in T- and B-lymphocytes improve survival following a cecal ligation and puncture, as well as prevent apoptosis in lymphoid organs (71). Transgenic mice overexpressing Bcl-2 in gut epithelial cells are also resistant to ischemia-reperfusion injury in the gut (72).

Therapies aimed at inhibiting lymphoid cell apoptosis may contribute to improved outcome, and should be considered in the treatment of hospitalized patients with sepsis syndromes. Although clinical trials with anti-apoptotic agents remain distant, largely due to technical difficulties associated with their administration and tissue targeting, inhibition of lymphocyte apoptosis may be an appropriate therapeutic target for the septic patient.

1.5. Role of dendritic cells during inflammation

Innate immunity focused on internal as well as exogenous signals has the ability to continuously discriminate between harmful and innocuous signals, as well as between self and nonself, and to generate an immune response only when required. The increasing complexity of the activation of the innate immune response assures that it is tightly regulated. All these mechanisms have an important role in preventing the systemic dissemination of microbial infection during its early phases while the adaptive (or acquired) immune response is being developed. The adaptive immune response is required for effective protection of the host against specific pathogenic microorganisms. The antigens of the pathogens are transported to local lymphoid

organs by migrating antigen-presenting cells (dendritic cells) **(Fig. 5)**. This antigen is processed and presented to antigen-specific naive T cells that continuously recirculate through the lymphoid organs. T-cell priming and the differentiation of armed effector T cells occurs here, and the armed effector T cells either leave the lymphoid organ to affect cell-mediated immunity at sites of infection in the tissues, or remain in the lymphoid organ to participate in humoral immunity by activating antigen-binding B cells. Which response occurs is determined by the differentiation of $CD4^+$ T cells into Th_1 (cell-mediated immunity) or Th_2 (humoral immunity) cells, which is in turn determined by the cytokines produced in the early innate or non-adaptive phase (14, 73, 74). $CD4^+$ T-cell differentiation is also affected by ill-defined characteristics of the activating antigen and by its overall abundance (75). Ideally, the adaptive immune response eliminates the infectious agent and provides the host with a state of protective immunity against re-infection with the same pathogen.

Dendritic cells (DC), which are located in many tissues and organs throughout the human body, are potent antigen-presenting cells (APC) and play a key role during the induction of immune response towards microbial pathogens (76). However, unlike macrophages, their major task is not to clear incoming pathogens but to alert the
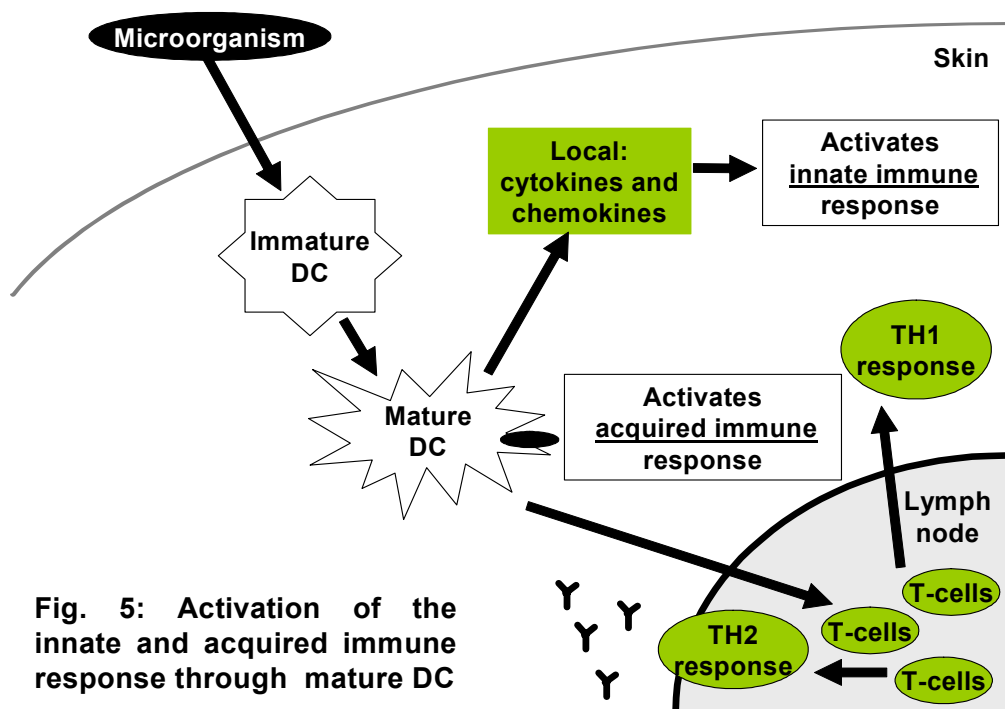

Fig. 5: Activation of the innate and acquired immune response through mature DC

immune system. Indeed, once activated by inflammatory stimuli and infectious agents, DC first produce chemokines and cytokines that activate or recruit macrophages, neutrophils, natural killer cells and immature DC at the inflammatory site, and then migrate to lymphoid organs in search of antigen-specific T cells **(Fig. 5)**. At the same time maturing DC upregulate receptors for lymphoid chemokines like CCR7, which drives DC migration to the lymph nodes, while on the other hand, they downregulte their inflammatory chemokine reseptors, such as CCR2 and CCR5 (77). While migrating to the lymph nodes, they shift from an endocytic/phagocytic immature state to a mature stage of efficient T cell stimulation. This irreversible process is named maturation and is accompanied by drastic morphological and functional changes. Only mature DC have high levels of surface expression of major histocompatibility complex (MHC) and costimulatory molecules that are essential for effective T cell stimulation (78).

Because DC, in large part, control the activation of B and T cells, they also control cytokine production. The magnitude of the cytokine response, determines the magnitude of the hyperinflammatory systemic inflammatory response syndrome (SIRS), as well as the development of sepsis induced multiorgan dysfunction or failure, sequentially leading to death. However, there is also increasing evidence to suggest that DC may actually modulate the increased lymphocyte apoptosis seen in sepsis syndrome and therefore would be an excellent target for gene therapy.

1.6. Gene therapy

Gene therapy represents a new modality for the treatment of acute and chronic diseases (79). Clinical trials are currently being performed in patients with hereditary diseases, such as cystic fibrosis (80) as well as infectious diseases (81, 82).

By definition, gene therapy means incorporating genes into a foreign cell by means of different non-viral or viral vectors **(Table 1)**. The host's machinery is then used to synthesize the gene-encoded protein locally. In contrast to this stands the direct, in most cases, systemic application of the protein product itself. The overall concept of gene therapy is formidable to deliver genes to cells that lack the specific gene or where the gene is abnormal and malfunctioning. As recent studies have shown this therapeutic approach may also benfit non-hereditary illnesses, such as

cancer and inflammatory diseases (HIV, hepatitis) (83-85). Brigham and colleagues have shown the advantages of gene therapy as a mode for drug delivery in acute and pulmonary diseases and confirmed its beneficial effect (84).

Over the last decade, more than 300 phase I and II gene therapy clinical trials have been investigating its efficiency in treating inherited or acquired genetic disorders as well as cancer (86-88). Despite the latest improvements reported in the area of vector design, both non-viral and viral based vectors **(Table 1)** are not yet sufficiently developed to allow application of gene therapy in phase III clinical trials.

Table 1: Non-viral and viral vectors

Non-viral vectors	Viral vectors
Naked DNA	Adenovirus
Liposome	Retrovirus
	Lentivirus
	Adeno-associated virus (AAV)
	Herpes simplex virus

Compared to traditional protein delivery, gene therapy has a variety of advantages for the treatment of acute inflammation. The first advantage is the ability to target specific tissues and insert genes without systemic effects. This is enabled by different routes of administration, such as applying intranasal or aerosoled vectors (89, 90). Furthermore, taking advantage of the natural tropism of vectors to target specific cells or using certain promoters will increase the therapy's specificity. For example, after intravenous injection of either adenovirus or adeno-associated virus expression of the transgene will primarily be in the liver (91). This route of administration is formidable to treat liver diseases, such as liver cancer or hepatitis (92). Furthermore, an attractive approach in gene therapy is that of using an inducible or repressible promoter system, like a tetracycline-regulated inducible promoter.

These promoters offer an additional regulating mechanism. Gene expression is then dependent on the application of the drug. This is very important when a constant expression of the gene is not desired (93, 94).

If duration of the therapy is desired for more than hours or even days, gene therapy has the advantage of a sustained expression of the gene leading to fewer or even only a single application, whereas conventional drug delivery is dependent on the drug's half-life **(Table 2)**. Protein-based therapies have half-lives of minutes to hours, a single gene delivery may last for a period of weeks or months, depending on the vector (95, 96).

Table 2: Characteristics of protein delivery (drug) and gene therapy

Drug	Gene therapy
half-life of minutes to hours	expression of days to weeks
less specific	highly specific
increased side effects	possibility of regulation
many applications	one or few applications

Gene therapy further leads to the opportunity of treating intracellular defects by transcribing proteins, which are physiologically not secreted by the cell. Not only is the protein then exactly transferred to its desired location, but side effects are also reduced.

In my opinion gene therapy is not a tool to permanently alter the genetic pattern of the host, but rather a novel approach to deliver and target protein based therapies. Gene therapy is and can be an effective tool to target the expression of protein based therapies in individual tissues. By modifying the vector and the promoter system, a high level of tissue specificity and regulation of expression can be achieved.

1.7. Adenovirus as a vector for gene therapy

Adenovirus is a linear, double stranded DNA virus surrounded by capsid proteins. By deleting specific regions of the virus genome, particularly the E1 region, and inserting a desired sequence under control of a constitutive viral promoter, the virus becomes a replication deficient vector capable of transferring exogenous DNA to differentiated, non-proliferating cells **(Fig. 6)**. Adenovirus has a large insertional capacity for foreign genes, in the range of 7-8kb. By deleting more of the adenoviral genome (usually the E3 or E4 regions) even larger DNA fragments can be inserted. Adenovirus is highly efficient in transfecting epithelial cells; it is common to see greater than 50% of a target cell population transduced. Due to these properties, adenovirus mediated gene therapy is very attractive for a variety of disorders, including various inflammatory diseases, cancer and neurologic disorders. However, there have been two intrinsic limitations to adenovirus that hinder its widespread use as a gene therapy vector: dose limiting toxicity and establishment of a cell-mediated and humoral immune response. The immunological and inflammatory response of the host to the introduction of adenovirus varies depending on the dose of the virus, the site of delivery and the generation of the virus, as well as the transgene being expressed (97). An intravenously administered adenovirus shows remarkable tropism for the liver, and primarily transfects hepatocytes (98). Although 90% of the virus is eliminated in the first 48 hours of infection through the innate immune response, as many as 95% of the hepatocytes are transfected after intravenous injection (99). Transgene expression generally lasts only 2-3 weeks with peak expression occurring during the first week (97). Loss of expression appears to be due, in part, to killing of virally infected cells, primarily through apoptotic processes. TNFα appears to play a major role in the clearance of the virus (100). Treatment of animals with a TNF binding protein (bp) results in prolonged gene expression mediated by adenovirus and reduces the magnitude of the inflammatory response (96). In addition, gene expression is transient because the adenovirus does not integrate its genome into the cellular host chromosomal DNA (101, 102). Therefore, both immunogenicity and the lack of adenoviral genome integration into the chromosomal DNA contribute to limited long-term transgene expression of adenoviral-mediated gene transfer. Taken together, these properties lead to the limitation of adenovirus as a vector for chronic

diseases, but makes it attractive for the treatment of acute inflammation, where an expression of one or two weeks is anticipated.

Immunogenicity derives from expression of adenoviral early genes (E1, E2, E3 and E4) (102). Deletion of the E1 region (first generation virus) is essential for generating replication-defective adenoviral vectors **(Fig. 6)** (103). New approaches are being pursued to decrease the immunogenicity: deletion of E2 and E4 regions in order to avoid expression of viral proteins within the transfected cells (104). Investigators are attempting to generate vectors deficient for all viral genes by constructing completely gutted vectors that contain only the viral terminal repeats and
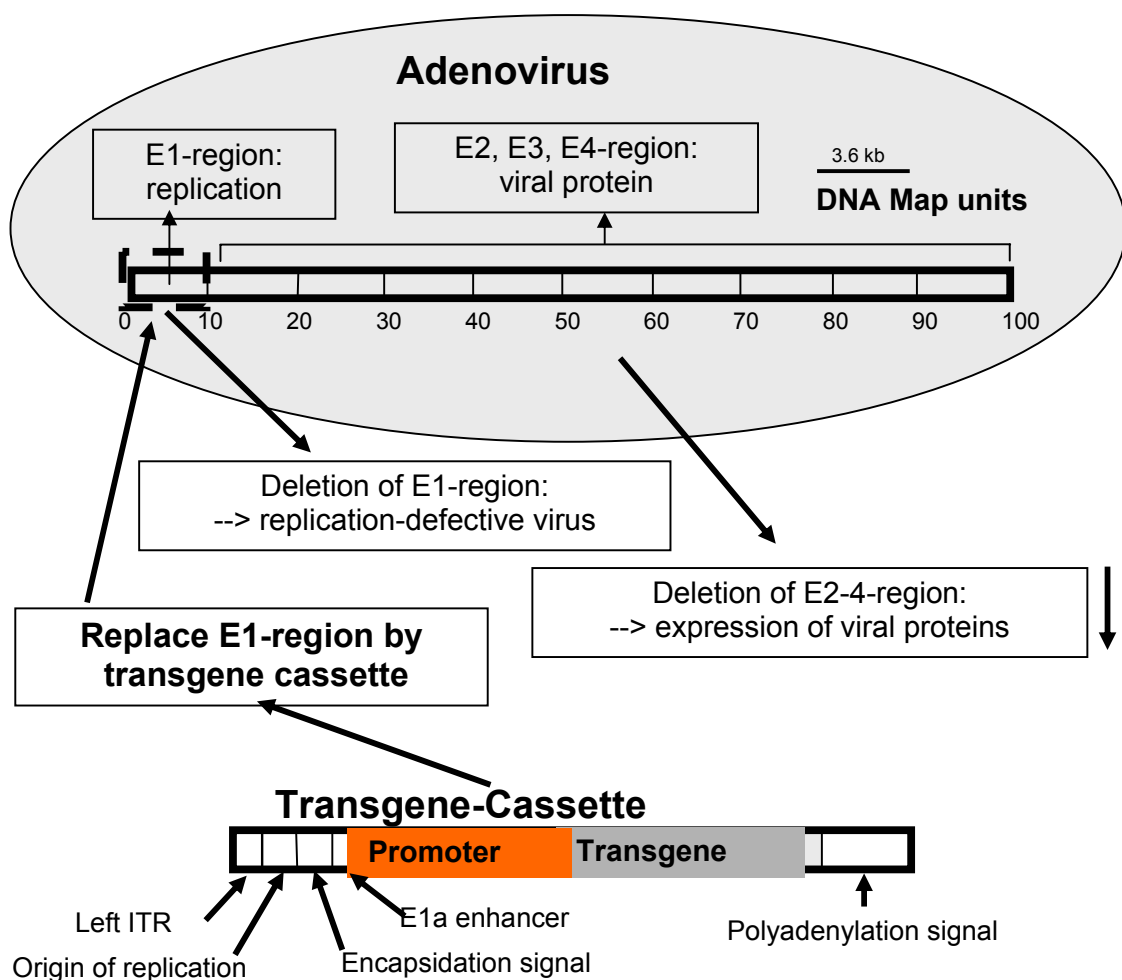

Fig. 6: First-generation adenovirus construct. Replication deficient adenovirus constructs are generated by deletion of either E1 and/or E3 regions and replacement with a cassette containing the promoter, transgene, and associated sequences. Deletion of the region determines the immunogenicity of the virus due to the transcribed viral proteins. The cassette containing the transgene can be inserted either in region E1 or E2-4.

the packaging sequence (105, 106). Deletion of not only the E1 region but also the E4 region of the adenovirus decreases expression of viral proteins, and reduces apoptosis of hepatocytes leading to blunted immunoreactions (107).

Due to the relative short duration of transgene expression, adenovirus appears to be a promising vector for transient gene therapy (173, 174), and appropriate for acute inflammatory processes, such as sepsis.

2. *Specific aims of the project*

The focus of my research in the recent years has been aimed at elucidating the pathophysiological mechanisms of the systemic inflammatory response syndrome in severely injured and septic patients (108-113).

Since treatment of sepsis and septic shock remains a clinical conundrum, and recent prospective trials aiming at modifying the inflammatory response have shown only modest clinical benefits, my interest has shifted towards therapies aimed at reversing the accompanying periods of immune suppression. Recent studies in experimental animals and critically ill patients have suggested that increased apoptosis of lymphoid organs and some parenchymal tissues may contribute to the immune suppression, anergy and organsystem dysfunction and appear to contribute to adverse mortality. Animal studies have demonstrated that blocking apoptosis can improve outcome in experimental models of severe sepsis. Although clinical trials with anti-apoptotic agents remain distant, mainly due to present technical difficulties associated with their administration and tissue targeting, inhibition of lymphocyte apoptosis represents an attractive therapeutic target for the septic patient.

In this light we focused on modifying the immune suppression by the help of gene therapy. We investigated the feasibility of gene therapy using an adenoviral construct for the treatment of acute inflammation induced by cecal ligation and puncture (114-117).