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Dynamic monitoring of monocyte HLA-DR expression for the diagnosis, prognosis, and prediction of sepsis

Yugang Zhuang[1], Hu Peng[1], Yuanzhuo Chen[1], Shuqin Zhou[1], Yanqing Chen[1]

[1]Emergency Department, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, No 301, Mid Yangchang Road, Shanghai, China

TABLE OF CONTENTS

1. ABSTRACT

Sepsis, an under-recognized health problem, is a major cause of death. More than 750,000 individuals develop sepsis annually, of whom 215,000 die of the disease. Clinical and experimental evidence indicates that patients with sepsis present with rapid impairment of immune function; biomarkers are therefore needed to enable early detection of this condition. Reduced monocyte human leukocyte antigen-DR (HLA-DR) expression, which is measured by flow cytometry, is currently the most popular biomarker for sepsis detection. In addition, the determination of HLA-DR expression provides valuable information in terms of predicting mortality and risk of secondary infections. HLA-DR levels have been shown to be inversely correlated with the severity of sepsis and immune dysfunction. In this review, we provide an overview of the association between sepsis and HLA-DR expression in terms of the predictive value of the latter in sepsis.

2. INTRODUCTION

Sepsis is among the ten leading causes of death in critically ill patients, and the third leading cause of death among patients in non-cardiac intensive care units (1, 2). More than 750,000 individuals develop sepsis annually, of whom 215,000 die of the disease (3). This high mortality rate has necessitated intensive research efforts aimed at elucidating the complex pathogenesis of sepsis, and applying the acquired knowledge to the development of immunomodulatory therapeutic interventions (4). Sepsis is considered largely an immunosuppressive disorder (5); in the absence of specific clinical symptoms, the diagnosis of immunosuppression currently depends on the assessment of paraclinical parameters. Although C-reactive protein (6), procalcitonin (7), angiopoietins (8), and other factors (9–11) have been used to predict sepsis, most of these lack specificity (12); it is therefore vital to develop reliable and effective biomarkers with higher specificity for the diagnosis of sepsis.

Volk *et al.* (13) were the first to describe immunoparalysis, which is indicated by human leukocyte antigen-DR (HLA-DR) expression on monocytes. HLA-DR has since proven useful for monitoring immunoparalysis, and is accepted as a reliable marker for evaluating immune function (14–17). Downregulation of HLA-DR has been associated with high mortality, and has shown predictive value for prognosis (18, 19) and risk of secondary infection in patients with sepsis (20, 21). Functional immunity, as assessed by HLA-DR expression, may reflect the net sum of pro- and/or anti-inflammatory influences, and therefore the actual immunological phenotype/ phase of sepsis. Importantly, as functional immunity involves dynamic processes, HLA-DR expression should not be considered a static index (14, 22-–24).

In this review, we discuss the physiological and clinical associations between HLA-DR expression and sepsis, and analyze the significance of HLA-DR as a biomarker for detecting sepsis.

3. SEPSIS AND THE IMMUNE RESPONSE: REDUCED EXPRESSION OF HLA-DR

Sepsis refers to the presence of a serious infection that correlates with systemic and uncontrolled immune activation (25, 26). This condition is characterized by a systemic inflammatory response syndrome (SIRS), which occurs due to injury and/ or infectious stimuli, in the presence of a known (or strongly suspected) infection. Patients with sepsis that exhibit evidence of organ dysfunction, including cardiovascular, renal, hepatic, or neurological impairment, are classified as having severe sepsis, while patients with cardiovascular dysfunction unresponsive to fluid are considered to be suffering from septic shock.

Sepsis is a syndrome rather than a disease (27). It often, but not exclusively, occurs in patients with infections. Up to 40% of clinical cases of sepsis occur as a result of sterile tissue injury arising from noninfectious sources such as pancreatitis, ischemia reperfusion injury, cancer, or numerous other disorders. The combination of variables that determines whether a patient with sepsis survives or succumbs is currently poorly understood (28); numerous patients who survive the initial critical phase of septic shock subsequently die as a result of secondary infections caused by pathogens that are normally only harmful in immunocompromised hosts (29, 30). A post-mortem study showed that patients who died in the intensive care unit following sepsis had biochemical, flow cytometric (FCM), and immunohistochemical findings consistent with immunosuppression (31).

The host immune response to sepsis is thought to be characterized by two sequential periods. An initial hyperinflammatory response that results in SIRS, referred to as a “cytokine storm”, is thought to be responsible for the high mortality and multiple organ dysfunction seen in septic syndromes (5, 32). The innate immune system releases proinflammatory cytokines and activates the complement and coagulation cascades to combat infection, whilst also recruiting members of the adaptive system to mount an intense immune response. This initial response is thought to be followed by a compensatory anti-inflammatory response syndrome (CARS), which is defined as a systemic deactivation of the immune system in order to restore homeostasis from an inflammatory state (33). Anti-inflammatory mediators may be released during this second period in an attempt to counteract continual inflammation. However, this process may be dysregulated, leading to persistent immune suppression and an increased risk of recurrent infections (34, 35). Recent data suggest that both the pro- and anti-inflammatory periods of the host immune response to severe injury and/or sepsis often occur concurrently (34).

HLA-DR, which is an HLA class II antigen, is a glycosylated cell-surface transmembrane protein that is expressed on antigen-presenting cells. HLA-DR is additionally constitutively expressed on monocytes such as macrophages, dendritic cells (DCs), and B cells. The expression of HLA-DR on monocytes (mHLA-DR) is essential for the presentation of peptides derived from ingested microbes to CD4-or CD8-positive T cells, thus initiating a specific immune response aimed at eliminating potential pathogens (16, 36). HLA-DR molecules, which are also required to activate helper T lymphocytes, play a central role in the specific immune response to infection.

Extensive research has demonstrated that reduced expression of HLA-DR occurs in patients with sepsis. The loss of mHLA-DR expression is an early event in sepsis, and the persistence of this alteration is associated with severity score, nosocomial infection, and death (37). The downregulation of mHLA-DR surface expression, measured by flow cytometry, has been postulated as a general biomarker of sepsis-induced immunosuppression, and as an independent predictor of nosocomial infection (20). The measurement of mHLA-DR expression has also been successfully applied to monitor the effectiveness of immunomodulatory therapies, including medications such as interferon-gamma (IFN-γ) (38), granulocyte/ macrophage colony-stimulating factor (GM-CSF) (39), thymosin alpha 1 (40), and filgrastim (41), as well as extracorporeal immune interventions such as immunoadsorption treatment and continuous hemodiafiltration (42, 43).

4. HLA-DR AS A BIOMARKER FOR SEPSIS

4.1. Evaluation of monocytic HLA-DR expression as a diagnostic and predictive biomarker for sepsis

The pioneering work of Polk and colleagues (44) in 1986 revealed an association between the occurrence of sepsis and low mHLA-DR expression. mHLA-DR expression was subsequently determined to be an effective prognostic marker in sepsis. Both the number of monocytes expressing HLA-DR and the density of HLA-DR expression have been shown to have prognostic value, and previous studies have reported on the accurate predictive use of low HLA-DR expression to identify patients most likely to die of sepsis (45). Cheron *et al*. (46) showed that mHLA-DR expression was decreased at day 1–2 post-trauma in all patients evaluated, with no difference in relation to the development of sepsis. However, a highly significant difference between septic and non-septic patients was observed at days 3-4, with non-septic patients showing increased mHLA-DR levels, whereas septic patients did not. Importantly, multivariate logistic regression analysis, after adjustment for usual clinical confounders, revealed that a slope of mHLA-DR expression, between days 1–2 and days 3–4, of < 1.2 was associated with the development of sepsis. These authors therefore concluded that monitoring immune function by measuring mHLA-DR should enable the identification of trauma patients at high risk of infection, with the slope of mHLA-DR recovery providing a significant predictor of subsequent sepsis. These studies support the notion that the recovery of normal mHLA-DR expression represents a critical point after injury.

Gouel-Cheron *et al.* (47) assessed mHLA-DR expression and IL-6 levels in 100 severely injured patients, and found that IL-6 levels and the slope of mHLA-DR expression between days 1–2 and days 3–4 differed significantly between septic and non-septic patients. Pradhan and colleagues (48) estimated a derived parameter, the “sepsis index” (SI), where SI = neutrophilic CD64 (nCD64)/mHLA-DR × 100 for the diagnosis and prognostication of neonatal sepsis. They found that the measurement of nCD64 levels at day 1 were useful for diagnosing neonatal sepsis, whereas measurement of mHLA-DR expression was beneficial for monitoring patients at later time points. The SI, which is a marker of moderate diagnostic sensitivity, supplements the current range of laboratory investigations used to detect neonatal sepsis. As a marker of prognosis, a high SI is associated with greater risk of mortality.

Overall, these studies demonstrate the predictive value of HLA-DR for sepsis, and suggest that the combination of an early marker of inflammation with mHLA-DR kinetics may provide a better biomarker of sepsis than the use of a single marker.

4.2. Value of monocyte HLA-DR expression as a biomarker for the development and prognosis of sepsis

Decreased mHLA-DR expression is now considered to represent a reliable marker of the development of immunosuppression and/or septic complications after trauma, surgery, pancreatitis, burns, and septic shock in critically ill patients (32, 49, 50). HLA-DR expression may be assessed in clinical practice using standardized tests (51, 52). Importantly, low levels of mHLA-DR have been observed in patients who subsequently developed nosocomial infections (20, 21, 46, 53). In contrast, mHLA-DR levels rapidly returned to normal (generally in < 1 week) in injured patients with uneventful recovery. Reduced mHLA-DR has thus been shown to predict adverse outcomes in various groups of critically ill patients (53). Patients with mHLA-DR expression of < 30% compared with the normal level exhibited a lower survival rate and 30-fold increased risk of mortality (18, 54).

However, not all studies have consistently identified low mHLA-DR as a significant indicator of poor prognosis; it is possible that low mHLA-DR expression may be associated with the CARS phase of sepsis rather than with a poor prognosis as such (55). Perry *et al.* (16) found that low monocyte surface expression and median fluorescence density of HLA-DR expression were not associated with high mortality and high Acute Physiology and Chronic Health Evaluation II (APACHE II) scores. Therefore, these authors consider the value of HLA-DR expression as a useful prognostic marker of outcome in sepsis controversial. The presence of low proportions of monocytes expressing HLA-DR may be indicative of CARS with an associated increased susceptibility to infection (56), rather than a direct indicator of outcome.

These apparently contradictory results highlight the need for a more representative index or system reflecting the connection between immune status of patients with sepsis and outcome of the disease.

4.3. Clinical significance of dynamic HLA-DR monitoring

Immune function changes dynamically during the clinical course of sepsis, suggesting that changes in HLA-DR expression exhibit similar trends. The apparent inconsistent findings with regard to the relationship between reduced HLA-DR expression and mortality in sepsis may be partially explained by the fact that immune function changes during the clinical course of severe sepsis.

Wu *et al*. (14) showed that single measurements of mHLA-DR within the first week after patient admission had no predictive value regarding mortality. In contrast, results expressed as dynamic parameters (i.e., differences between two time points) provided excellent predictive values, especially the difference in mHLA-DR expression between days 0 and 3 or days 0 and 7. Importantly, multivariate analysis showed that these two parameters remained the sole independent predictors of mortality, with a significantly elevated odds ratio. The authors proposed that the dynamic changes in mHLA-DR over time serve as a better predictor of mortality than a single value at any given time point. Gouel-Cheron *et al*. (47) also underlined the utility of daily monitoring of immune function to identify trauma patients at high risk of infection. These results underscore the importance of dynamically monitoring immune function in patients at high risk of sepsis in order to identify those most likely to develop sepsis, assess their severity, and evaluate their prognosis.

5. MECHANISMS OF HLA-DR-INDUCED IMMUNOSUPPRESSION AND SEPSIS

5.1. Mechanisms underlying reduced HLA-DR expression

Although numerous researchers have shown that low monocyte surface expression of HLA-DR is a characteristic of sepsis, the mechanisms underlying this phenomenon remain unclear. Possible mechanisms include downregulation of gene transcription resulting in reduced mRNA production, or post-translational modification of HLA-DR, which may affect translocation to the cell surface (57). Alternatively, HLA-DR may be released from the monocyte surface and shed into the circulation in a soluble form (sHLA-DR): increased levels of sHLA-DR have been detected in the plasma and synovial fluid in hyperinflammatory states (58), and *in vitro* experiments have demonstrated that the inflammatory cytokine IFN-γ induces shedding of HLA-DR by human monocytes (59).

The regulation of HLA-DR at the level of gene transcription has been investigated in patients with sepsis. The regulation of HLA-DR is correlated with high cortisol levels, which are thought to downregulate HLA-DR gene transcription by lowering the levels of MHC class II transactivator A (37). In addition, IFN-γ has been shown to regulate HLA-DR gene transcription in melanoma cell lines (60). Re-endocytosis and intracellular sequestration of MHC class II molecules has also been analyzed in relation to decreased HLA-DR surface expression (61). Furthermore, granulocyte macrophage colony-stimulating factor (GM-CSF) has been shown to increase shedding of HLA-DR from the cell surface and increase HLA-DR gene transcription in patients with sepsis (57).

Precise details of the mechanism underlying the downregulation of HLA-DR in sepsis thus remain unclear, and further studies are needed to elucidate this relationship.

5.2. Monitoring changes in innate immunity

Sepsis is accompanied by disorders of both the innate and adaptive immune systems. Changes, such as apoptosis of CD4-lymphocytes and B lymphocytes and immunoparalysis of monocytes, are recognized features in patients with sepsis (62–65).

Innate immune cells represent the first line of defense following infection, and thus play a central role in the control of pathogens and initiation of adaptive immune responses. DCs play a critical role in the initiation and modulation of T-cell responses, and exhibit altered phenotypes and functions in sepsis such as decreased expression of HLA-DR and reduced secretion of pro-inflammatory cytokines upon stimulation by bacterial products (66, 67).

The reduced expression of HLA-DR on circulating monocytes has been proposed as a surrogate marker of failure of the innate immune response (68). Endotoxin tolerance in monocytes and macrophages increases the release of immunosuppressive mediators, mainly IL-10, and decreases antigen presentation through decreased HLA-DR expression. Both of these factors are associated with poorer outcomes in patients with sepsis (69, 70). Continued release of IL-10 may contribute to, or amplify, sepsis-induced immunosuppression, thereby increasing susceptibility to secondary microbial infections. Several studies have demonstrated an association between low mHLA-DR expression and impaired monocyte function, e.g. in terms of reduced secretion of tumor necrosis factor (TNF) and IL-1β in response to bacterial challenge (71) and decreased lymphocyte proliferation in response to tetanus toxin, presumably as a result of impaired antigen presentation (72).

Therapeutic protocols including the administration of GM-CSF and IFN-γ have been utilized in patients with sepsis, with the aim of stimulating innate immune function, improving myelopoiesis, and limiting lymphocyte apoptosis (73). It has been shown that, along with the recovery of mHLA-DR expression, TNF-α release in stimulated whole blood is restored in response to immunotherapy (74).

5.3. Monitoring changes in adaptive immunity

Since the discovery of the association between loss of delayed-type hypersensitivity response in surgical patients and increased risk of nosocomial infections, T lymphocyte anergy has been shown to represent a hallmark of sepsis-induced

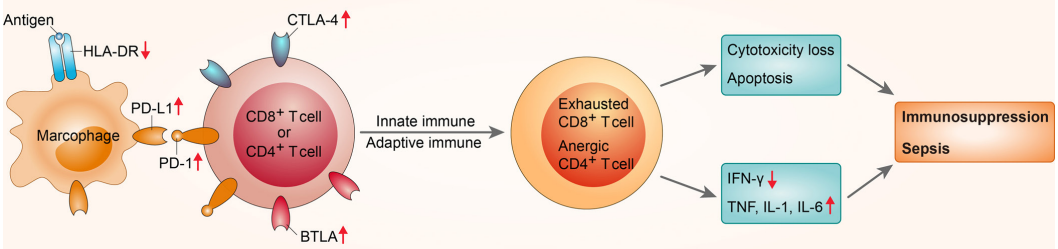

Figure 1. Schematic of immunosuppression in sepsis. Immunosuppression of sepsis is mediated by multiple mechanisms including decreased expression of the cell surface antigen presenting complex HLA-DR, and increased expression of the negative co-stimulatory molecules programmed death 1 (PD-1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), B and T lymphocyte attenuator (BTLA), and their corresponding ligands, such as PD-L1, resulting a compromised innate and adaptive immune system with poorly functional "exhausted" CD8- and anergic CD4-positive T cells, and inducing T cell apoptosis and cytotoxicity loss, decreasing IFN-γ production, and increased pro-inflammatory cytokine (e.g., TNF, IL-1, IL-6) release.

immunodysfunction (75, 76). In particular, markedly decreased cell numbers associated with increased apoptosis are consistently found in patients (77, 78). In addition, functional alterations, such as reduced proliferation as well as decreased pro-inflammatory (IL-2, IL-17, IFN-γ) and increased anti-inflammatory (IL-10) cytokine production, have been observed (31, 79, 80). Phenotypic alterations, such as reduced expression of co-activating receptor CD28 and increased expression of co-inhibitory receptor PD-1 or CTLA4, or reduced diversity of the T-cell receptor repertoire, also represent characteristics of T lymphocytes in sepsis (81-83). Further, an increased percentage of circulating CD4+CD25+ regulatory T cells has been observed in patients with sepsis (84) (Figure 1). However, with the exception of mHLA-DR measurement, no consensus on markers of sepsis-induced lymphocyte alterations has emerged to date. The best currently available test for assessing lymphocyte functionality remains the measurement of proliferation in response to recall antigens or mitogen stimulation (73). Major efforts are therefore needed to develop robust biological tests of lymphocyte function that correlate with objective outcomes of survival in sepsis.

6. MEASUREMENTS OF HLA-DR EXPRESSION

6.1. Flow cytometry

Monocytes strongly express HLA-DR on their surface, thereby enabling facile detection by FCM. HLA-DR expression on monocytes may be measured either as the percentage of cells positive for this marker, or as the mean fluorescence intensity of the marker among all monocytes (85). FCM is of potential utility at each step of management of patients with sepsis, from the diagnosis of infection to the design of targeted individualized therapy and optimization of drug efficacy.

However, FCM has important practical limitations, including variability of results arising from differences in specimen handling, the need for immediate analysis of blood samples without storage, and the consequent exclusion of samples from health-care units without FCM facilities (86). These limitations of traditional mHLA-DR monitoring techniques clearly hamper their clinical utility, reducing the feasibility of large multi-center studies.

6.2. Quantitative reverse transcription–polymerase chain reaction

The application of quantitative reverse transcription–polymerase chain reaction (qRT–PCR) to measure HLA-DR expression has been suggested as a novel alternative to FCM for identifying immunosuppression in sepsis (86). Patients with sepsis demonstrate reduced surface expression of mHLA-DR as well as reduced mRNA levels. The mRNA levels of HLA-DRA, which encodes the non-polymorphic region of the α-chain of the HLA-DR molecule, in whole blood were shown to correlate highly with surface expression of HLA-DR when monitored by qRT–PCR. This finding indicates that HLA-DRA expression may serve as a useful biomarker for evaluating immunosuppression in sepsis.

However, the evaluation of HLA-DR by qRT–PCR may combine the levels from monocytes, DCs, B lymphocytes, and activated T cells, whereas FCM enables specific measurement of levels from monocytes. qRT–PCR is therefore not yet a suitable replacement for FCM as a means of detecting HLA-DR expression. Further improvements in qRT–PCR technology should be monitored and applied for the development of standardized and automated protocols for the assessment of HLA-DR expression.

7. CONCLUSIONS

Sepsis remains an under-recognized health-care problem and a leading cause of death. There is an urgent need to identify and develop indicators to predict the occurrence, outcome, and prognosis of sepsis. The detection of monocyte expression of HLA-DR by FCM may serve as a good prognostic indicator in most patients with sepsis, and has already been

applied in critically ill patients. Furthermore, dynamic monitoring of HLA-DR, which is representative of changes in immune function, may provide a more accurate predictive tool than static monitoring. Owing to the complex pathophysiology of sepsis, success on the diagnosis of sepsis will be better attained by not just looking at one particular biomarker but more likely a combination of several biomarkers. The combination may help overcome the limitations of sensitivity and specificity that single biomarker readouts have routinely shown.

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Abbreviations: HLA-DR, human leukocyte antigen-DR; PD-1,programmed death-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen-4; BTLA, B and T lymphocyte attenuator; IFN-γ, interferon gamma; TNF, tumor necrosis factor; IL, interleukin.

Key Words: Sepsis, Human Leukocyte Antigen DR, HLA-DR, Immunosuppression, Review

Send correspondence to: Yanqing Chen, Emergency department, Shanghai Tenth People's hospital, Tongji University of Medicine, North ZhongshanRoad, Shanghai 200003, China. Tel: 021-66300588; Fax: 021-66300588; E-mail: yanqing_Chen@yeah.net