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Messenger RNA expression of major histocompatibility complex class II genes in whole blood from septic shock patients*

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Objective: The decreased expression of human leukocyte antigen (HLA)-DR on monocytes is proposed as a major feature of sepsis-induced immunodepression. The objective of the present study was to investigate, in whole blood from septic shock patients, the messenger RNA (mRNA) expression of a gene panel, which is essential to ensure major histocompatibility complex class II protein structure, transport, and peptide loading.

Design: The authors conducted a cohort study.

Setting: This study was conducted in intensive care units at a university hospital.

Subjects: The study included septic shock patients (n = 41) and healthy volunteers (n = 15).

Measurements and Main Results: Using quantitative reverse transcriptase–polymerase chain reaction, we found that the highly polymorphic HLA-DRB1 and nonpolymorphic-DRA mRNA levels were significantly decreased in whole blood from patients with septic shock compared with healthy volunteer both on days 1–3 and 4–10 after the onset of shock. This profile was also observed for genes encoding the invariant chain, the transcription factor class II transactivator (CIITA), and the enzymes involved in the peptide loading cathepsin S, HLA-DMA, and -DMB. The monocyte surface expression of HLA-DR measured by flow cytometry

was significantly correlated with the whole-blood mRNA levels of HLA-DRB1 and -DRA and to a lesser extent with the other CIITA-regulated genes HLA-DMA and invariant chain. The correlation between HLA-DRB mRNA and cell-surface expression levels was also observed in a small subset of purified monocyte samples. Regarding the temporal relationship, a significant increase of whole-blood HLA-DRA, -DMA, -DMB, invariant chain, and CIITA mRNA level was observed in survivors ($p = .001$), whereas expressions remained low in nonsurvivors.

Conclusions: A global transcriptional down regulation of a gene panel required for MHC II-restricted antigen presentation may occur in the course of septic shock. Our results suggest that the transcriptional resumption of CIITA-regulated genes might contribute to the recovery of membrane HLA-DR expression observed in survivors. These results obtained at the mRNA level support previous reports describing the loss of monocyte HLA-DR at the protein level and thus confirm the potential of measuring monocyte HLA-DR in septic patient followup. (Crit Care Med 2005; 33:31–38)

KEY WORDS: human leukocyte antigen-DR; sepsis; blood; messenger RNA; reverse transcriptase–polymerase chain reaction; class II transactivator

Despite >20 yrs of extensive research, severe sepsis and septic shock remain the leading causes of mortality in critically ill patients (1). Over the past 2 decades, the results of clinical trials using

antiinflammatory therapies have been disappointing (2, 3). Although the host response to an infection consists in a quasi-simultaneous release in the blood of pro- and antiinflammatory mediators, the systemic antiinflammatory response rapidly predominates in many patients (4). This sustained immunosuppressive profile, called “immunoparalysis,” is primarily characterized by T-cell anergy and a defect in antigen presentation (5, 6). Although the direct link between immunoparalysis and sepsis-associated morbidity and mortality has not been clearly demonstrated thus far, it is well established that critically ill patients with persistent immunoparalysis show an increased risk of contracting a nosocomial infection (7, 8), which might dramatically worsen their prognosis. The failure of antiinflammatory treatments appears to be the result of, at least in part, the lack of tools allowing a precise characterization and staging of patients (3).

The decreased expression of human leukocyte antigen-DR (HLA-DR) on monocytes constitutes a reliable marker of immunoparalysis (9, 10) and seems to correlate with an increased risk of a fatal outcome (11–13). Immune monitoring by studying monocyte HLA-DR expression might therefore help to guide medical decision making (12, 14). Hence, understanding the molecular basis of immunoparalysis represents an important challenge to establish causal therapies and prevention strategies for this clinical situation. The decreased expression of HLA-DR in monocytes observed in the course of septic shock remains poorly understood. The reendocytosis and intracellular sequestration of major histocompatibility complex class (MHC) II molecules was recently described in purified monocytes (15). This phenomenon appears to be mainly mediated by interleukin 10, which plays a major role in sep-

*See also p. 236.

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sis-induced immunoparalysis (15). Moreover, *in vitro* studies have demonstrated that the simultaneous interference of multiple mechanisms is behind the reduction of MHC II expression in experimental endotoxin tolerance (16). This monocyte deactivation after an acute response to lipopolysaccharide has been proposed as a valuable model of postinflammatory immunodeficiency (10, 12). Among the different mechanisms evoked, a marked transcriptional down regulation of the genes involved in the MHC II process was observed, which may be related to the reduced expression of the transcription factor class II transactivator (CIITA) (16).

The first requirement of HLA-DR expression on cells is the synthesis of the nonpolymorphic α and highly polymorphic β chains. Then, the biosynthesis and transport of MHC II involves various accessory molecules such as chaperon proteins and enzymes. The genes encoding these major components of the antigen processing and presentation pathway are tightly and coordinately regulated at the level of transcription as illustrated by extensive research performed in patients with bare lymphocyte syndrome, a hereditary immunodeficiency resulting from the absence of MHC II expression (17).

The aim of our study was to investigate the expression of MHC II genes in septic shock by measuring in whole blood the messenger RNA (mRNA) levels of a gene panel, which is essential to ensure MHC II protein structure, transport, and peptide loading.

MATERIALS AND METHODS

Study Design. Forty-one consecutive patients with septic shock admitted to the surgical or medical intensive care units of a university hospital were enrolled in the study. This prospective study was approved by the local ethics committee, which waived the need for informed consent. Septic shock was defined according to the consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine (18). Severity was assessed using the Simplified Acute Physiology Score (SAPS) II (19). Because it is essentially the restoration after day 4–5 of HLA-DR expression on monocytes that was found to have a good prognostic value (13, 20), a first blood sample was collected early after the onset of shock (<72 hrs) and, when possible, a second one later in the course of the syndrome (>72 hrs). Mortality was defined as death occurring within 28 days after the onset of septic shock. Fifteen healthy volunteers (mean age, 56 ± 4 yrs; male:female ratio,

1:25), enrolled regularly during the study, served as controls.

Flow Cytometry and DNA Extraction. HLA-DR expression on monocytes was assessed by using flow cytometry (EPICS XL; Beckmann-Coulter) as previously described (13). Genomic DNA was extracted from the cell pellet using a standard phenolic extraction protocol as previously described (21).

RNA Extraction from Purified Monocytes and Whole-Blood Samples. For a random subset of patients ($n = 6$) and healthy donors ($n = 3$), monocytes were studied from blood samples collected in ethylenediaminetetraacetic acid tubes. Monocytes were positively isolated (anti-CD14 antibodies, isotype: mouse immunoglobulin-G2a, clone TUK4; Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells using a magnetic cell separation system (MACS; Miltenyi Biotec) with a cell purity higher than 90%. Total RNA was isolated from the purified monocytes using TRIzol Reagent (Invitrogen, Carlsbad, CA) and treated with DNase (Ambion, Austin, TX).

The whole-blood mRNA expression profile was studied using blood samples collected directly in PAXgene blood RNA tubes (PreAnalytix, Hilden, Germany). Total RNA was extracted using the PAXgene Blood RNA kit (PreAnalytix). Before RNA elution, the residual genomic DNA was digested using the RNase-Free DNase set (Qiagen, Hilden, Germany).

Total RNA was reverse transcribed into complementary DNA (cDNA) using the ThermoScript reverse transcriptase-polymerase chain reaction system (Invitrogen) according to the manufacturer's instructions. The mRNA expression of genes required for MHC II-restricted antigen presentation (HLA-DRA, -DRB, -DMA, -DMB, invariant chain [Ii], cathepsin S) and CIITA was quantified using q-reverse transcriptase-polymerase chain reaction. PCR reactions were performed in a LightCycler instrument using the Fast-Start DNA Master SYBR Green I real-time PCR kit according to the manufacturer's instructions (Roche Molecular Biochemicals, Basel, Switzerland). Thermocycling was performed in a final volume of 20 μ L containing 3 mM $MgCl_2$ and 0.5 μ M each of the required primers. PCR was performed with an initial denaturation step of 10 mins at 95°C, followed by 40 cycles of a touchdown PCR protocol (10 secs at 95°C, 10 secs annealing at 68–58°C, and 16 secs extension at 72°C).

The cDNA calibrators were prepared

from purified PCR amplicons obtained with the corresponding primers (Table 1). To control the PCR specificity, PCR products were sequenced and matched to the GeneBank database. Expression of the housekeeping gene PPIB encoding for cyclophilin B was investigated using specific cDNA standards and ready-to-use primer mixes obtained from Search-LC (Heidelberg, Germany). The efficiency of using PPIB mRNA levels as a reference for target mRNA quantification in human peripheral blood was previously studied at our institute (25).

The Second Derivative Maximum Method was used with the LightCycler software to automatically determine the crossing point for individual samples as previously described (26). Serial dilutions of each cDNA standard were prepared in quadruplicate to generate standard curves. Relative standard curves describing the PCR efficiency of selected genes and PPIB were created and used to perform efficiency-corrected quantification with the LightCycler Relative Quantification Software (Roche Molecular Biochemicals).

mRNA Quantification of HLA-DRB1 Alleles. To take into account the interindividual allelic variation of HLA-DRB1, specific primers (Table 1) were designed according to the patient's DRB1 genotype (HLA-DR oligo-detection kit bioMérieux, Marcy l'Etoile, France) (27) to perform q-reverse transcriptase-polymerase chain reaction. Forward DRB1 primers annealed to the first hypervariable region of exon 2. Reverse primer annealed to a conserved region of DRB genes located between the second and third hypervariable regions of exon 2. In each heterozygous individual, both DRB1 alleles were tested. The overall number of HLA-DRB1 mRNA copies corresponding to both alleles was normalized to the number of PPIB mRNA copies.

Statistical Analysis. The comparison between groups was performed with the nonparametric Mann-Whitney U test. As a result of the lack of serial data for the healthy donors, the groups were compared only once using the earliest measurement for each septic patient, which was obtained during the first 72 hrs after the onset of septic shock.

The nonparametric Wilcoxon's paired test was used to assess time-related individual variation of the parameters between the two observation periods. Among the 41 patients enrolled in the study, nine patients could not be in-

Table 1. Design of primers used for the messenger RNA quantification of transcription factor class II transactivator (CIITA) and genes required for major histocompatibility complex II-restricted antigen presentation using real-time polymerase chain reaction

Gene	Accession No.	Sequence
HLA-DRA	NM_019111	5'-GCCAACCTGGAAATCATGACA-3' (sense) (22) 5'-AGGGCTGTTTCGTGAGCACA-3' (antisense) (22)
CIITA	NM_000246	5'-CCTGCTGTTTCGGGACCTAAA-3' (sense) (22) 5'-GGATCCGCACCAGTTTGG-3' (antisense) (22)
Invariant chain	NM_004355	5'-GCAGAGGCGTCTTCAACA-3' (sense) (15) 5'-GCACCAGGTCTCATGGGAT-3' (antisense)
Cathepsin S	NM_004079	5'-CAATAAAGGCCAGTGTCTGTTG-3' (sense) (15) 5'-GATGGTTCATAGTAGACACCACTTCTG-3' (antisense) (15)
HLA-DMA	NM_006120	5'-CTGTGTGGCAAGAAGGTATGGGT-3' (sense) (23) 5'-CATTGGAGTAGGAGCTTCAGGGA-3' (antisense)
HLA-DMB	NM_002118	5'-GGCATCTTTACAGAGCAGAGCAT-3' (sense) (23) 5'-ATGTGAAATCCTTTGGAGTCCCA-3' (antisense) (23)
HLA-DRB1*01		5'-GGCAGCTTAAGTTTGAATG-3' (sense) (24) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense) (24)
HLA-DRB1*03, *08, *11, *12, *13, *14		5'-AGTACTCTACGTCTGAGTG-3' (sense) (24) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense) (24)
HLA-DRB1*04		5'-AGCAGGTTAAACATGAGTG-3' (sense) (24) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense) (24)
HLA-DRB1*07		5'-CTGTGGCAGGGTAAGTATA-3' (sense) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense)
HLA-DRB1*08, *11, *12, *14		5'-AGTACTCTACGGGTGAGTG-3' (sense) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense)
HLA-DRB1*15, *16		5'-CTAAGAGGGAGTGCATTT-3' (sense) 5'-AGGCCGCCCCAGCTCCGT-3' (antisense)
HLA-DRB		5'-AGTACCGGGCGGTGACGGAG-3' (sense) 5'-TCGCCGCTGCACTGTGAAG-3' (antisense)

HLA, human leukocyte antigen.

cluded in this analysis either because they deceased or left the intensive care unit before the second sample or because the mRNA quantification was not successful for the all-gene panel.

Correlation between parameters was studied with the Spearman's rank-correlation test. We considered $p < .05$ to be significant without correction of the number of tests performed. Box and whisker plots, with representation of the median, 75th and 90th percentiles, and outliers, were used for most of the graphic representation.

RESULTS

Patients. The global mortality rate was 35%. Mean SAPS II admission score was significantly increased in nonsurvivors compared with survivors. None of the other clinical and demographic characteristics described in Table 2 displayed significant differences between the two groups.

Surface Expression of HLA-DR on Monocytes. During the first observation period (first 72 hrs after the onset of septic shock), compared with healthy donors ($n = 15$), the expression of HLA-DR on monocytes was severely and significantly reduced in septic shock patients ($n = 41$) without overlap ($p < .001$). Pa-

tients who survived showed a higher percentage (Fig. 1) and mean fluorescence intensities (data not shown) of HLA-DR-positive monocytes than patients who did not, both in the early (survivors: $n = 27$ vs. nonsurvivors: $n = 14$) and late (survivors: $n = 24$ vs. nonsurvivors: $n = 10$) phases of septic shock. Comparing the periods of days 1–3 and 4–10, a significant increase of monocytic HLA-DR expression was observed in survivors ($p = .001$, Wilcoxon's paired test) but not in nonsurvivors. Regarding the number of circulating monocytes, no significant differences were observed between survivors and nonsurvivors both at the early and late phase of the septic shock, and no significant variations were observed over time in each group (survivor days 1–3: 556 ± 367 ; nonsurvivors days 1–3: 720 ± 454 ; survivor days 4–10: 674 ± 333 ; nonsurvivors days 4–10: 881 ± 495 monocytes/ μ L).

HLA-DRB mRNA Level in Purified Monocytes Is Highly Correlated with Surface Expression. The expression of HLA-DRB mRNA, including DRB1 alleles and, for most HLA haplotypes, those of the second DRB locus expressed (i.e., DRB3, DRB4, or DRB5), was measured using DRB generic primers in purified monocytes from six patients with septic

shock (three survivors and three nonsurvivors; median SAPS II score: 65; median sampling time: day 6) and three healthy volunteers. After monocyte purification, a fraction of cells was also used to measure the HLA-DR surface expression on monocytes by flow cytometry. The HLA-DRB mRNA expression in purified monocytes was systematically lower in septic shock patients than in healthy volunteers (Fig. 2). Moreover, HLA-DRB mRNA level was correlated both with the percentage ($r = .9$; $p < .01$) and the mean fluorescence intensities ($r = .87$; $p = .01$) of HLA-DR-positive monocytes.

HLA-DRB1 and -DRA mRNA Expression in Whole Blood. Based on the HLA-DRB1 genotype determined in patients and controls, DRB1 allele-specific mRNA was quantified in whole-blood samples obtained from a random subset of 11 healthy volunteers and the first 19 septic shock patients (nine survivors and ten nonsurvivors) enrolled prospectively in the study. The overall DRB1 mRNA was significantly decreased in septic shock patients compared with healthy volunteers ($p < .0001$) (Fig. 3). When survivors and nonsurvivors were compared, there was no difference in the DRB1 mRNA level in the early phase of septic shock (days 1–3). However, when measured later in the

Table 2. Demographic, clinical characteristics, microbiology, and type of infection

	Survivors n = 27 (%)	Nonsurvivors n = 14 (%)	Total n = 41 (%)	<i>p</i>
Male	17 (63)	8 (57)	25 (61)	.717
Female	10 (37)	6 (43)	16 (39)	
Age, yrs ^a	63 (45–73)	68 (58–79)	65 (52–74)	.141
Length of stay in the intensive care unit	11 (8–19)	8 (5–19)	10 (6–20)	.230
SAPS II (19) at admission ^a	45 (39–57)	66 (60–70)	51 (43–64)	.0002
Microbiologically documented diagnosis	20 (74)	11 (79)	31 (76)	
Bacilli Gram (–)	12 (44)	7 (50)	19 (46)	.735
Cocci Gram (+)	12 (44)	8 (57)	20 (48)	.440
Fungi	5 (19)	5 (35)	10 (27)	.224
Type of infection				
Community-acquired	10 (37)	6 (43)	16 (39)	.717
Nosocomial (28)	17 (63)	8 (57)	25 (61)	
Site of infection				
Pulmonary	12 (44)	8 (57)	20 (49)	.667
Abdominal	11 (41)	5 (36)	16 (39)	
Others	4 (15)	1 (7)	5 (12)	

SAPS, Simplified Acute Physiology Score.
^aMedian (Q1–Q3).

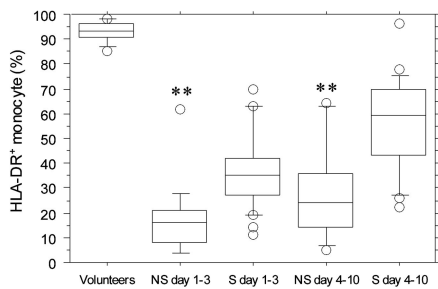


Figure 1. Percentage of human leukocyte antigen (HLA)-DR-positive monocytes in peripheral blood of survivor (S; n = 27), nonsurvivor (NS; n = 14) septic shock patients and healthy volunteers (n = 15) measured by flow cytometry. Septic shock patients were analyzed during the first 72 hrs after the onset of shock and later between days 4 and 10. ***p* < .01 vs. survivors, Mann-Whitney U test.

syndrome (days 4–10), the DRB1 mRNA expression was significantly higher in survivors (*p* = .02), suggesting an ongoing recovery of the HLA-DRB transcription activity.

Moreover, both in the early and late phase of septic shock, we observed a major and significant down regulation of DRA mRNA expression in whole blood compared with control values (*p* < .0001) (Fig. 4A). During both observation periods, DRA mRNA expression was higher in the survivor than in the nonsurvivor group. The difference reached statistical significance only at days 4–10.

Both HLA-DRA and DRB1 mRNA levels measured in whole blood showed a significant correlation with the percentage (HLA-DRA: *r* = .74, *p* < .0001; HLA-DRB1: *r* = .72, *p* < .0001) or mean fluorescence intensities (data not shown) of

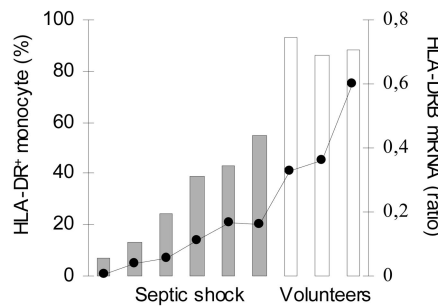


Figure 2. Cell surface expression of human leukocyte antigen (HLA)-DR measured by flow cytometry (bars) and HLA-DRB messenger RNA (mRNA) level measured by q-reverse transcriptase–polymerase chain reaction (line) in purified monocytes from healthy volunteers (n = 3) and patients with septic shock (n = 6). The HLA-DRB mRNA level was normalized to that of the housekeeping gene PPIB. The cell surface HLA-DR expression corresponds to the percentage of HLA-DR⁺ monocytes.

HLA-DR⁺ monocytes measured by means of flow cytometry.

mRNA Expression of MHC-II Genes, CIITA, and Cathepsin S in Whole Blood. MHC II α/β heterodimers initially assemble in the endoplasmic reticulum with Ii, which acts as a chaperone to stabilize the heterodimer and targets class II molecules to late endosomal compartments (29). In the present study, we found a decreased expression of Ii mRNA in septic shock (*p* < .0001), especially in nonsurvivors (Fig. 4B).

After reaching the endosomal compartment, Ii is partially released from the complex, leaving a residual peptide named class II invariant chain peptide. The generation of class II invariant chain

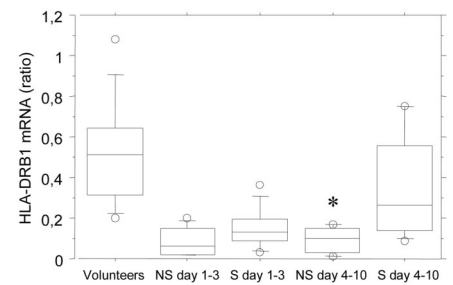


Figure 3. Human leukocyte antigen (HLA)-DRB1 messenger RNA (mRNA) level in peripheral blood of survivors (S; n = 9) and nonsurvivor (NS; n = 10) septic shock patients and healthy volunteers (n = 11) measured by q-reverse transcriptase–polymerase chain reaction. Primers were selected according to the HLA-DRB1 genotype. Overall HLA-DRB1 mRNA level, corresponding to both alleles, was normalized to that of the housekeeping gene PPIB. Septic shock patients were analyzed during the first 72 hrs after the onset of shock and later between days 4 and 10. **p* < .05 vs. survivors, Mann-Whitney U test.

peptide is mediated by cysteine endopeptidases and especially cathepsin S. In our study, we found a reduced expression of cathepsin S mRNA in the overall population of septic shock compared with healthy volunteers (*p* < .0001). In the early phase of septic shock, the cathepsin S mRNA level did not differ between patients who died and those who survived, whereas it was significantly increased in survivors in the late observation period (days 4–10) (Fig. 4C).

The release of class II invariant chain peptide and its replacement with antigenic peptides is catalyzed by HLA-DM (29). Compared with control values, septic shock was characterized by a down

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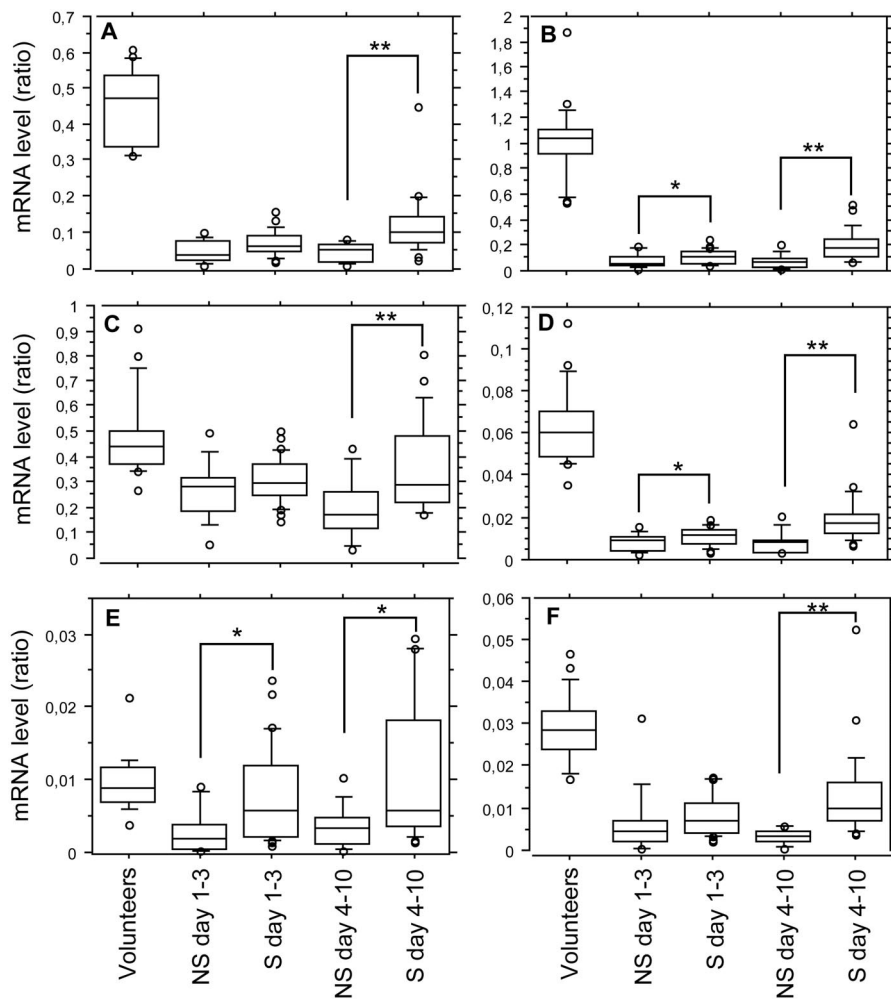


Figure 4. Messenger RNA (*mRNA*) level of human leukocyte antigen (HLA)-DRA (A), invariant chain (B), cathepsin S (C), HLA-DMA (D), -DMB (E), and class II transactivator (F) in peripheral blood of survivor (S; $n = 27$) and nonsurvivor (NS; $n = 14$) septic shock patients and healthy volunteers ($n = 15$) measured by q-reverse transcriptase–polymerase chain reaction. The mRNA level was normalized to that of the housekeeping gene PPIB. Septic shock patients were analyzed during the first 72 hrs after the onset of shock and later between days 4 and 10. * $p < .05$ vs. survivors; ** $p < .01$ vs. survivors, Mann-Whitney U test.

regulation of HLA-DM mRNA, especially the HLA-DMA isoform ($p < .001$). The expression of both isoforms was significantly lower in nonsurvivor patients (Fig. 4, D and E).

The expression of Ii, HLA-DMA, -DMB, -DRA, and DRB genes are known to be coordinately regulated at the level of transcription by a conserved set of factors, of which CIITA has a central role (29). Here, we found a marked down regulation of CIITA mRNA expression in septic shock compared with control values ($p < .0001$), especially in nonsurvivors (Fig. 4F).

The correlation analysis showed a significant correlation between whole-blood HLA-DRA, -DMA, Ii, and CIITA mRNA levels (Table 3). Moreover, HLA-DMA and

Ii mRNA levels showed a trend toward a correlation with the percentage (HLA-DMA: $r = .70$, $p < .0001$; Ii: $r = .65$, $p < .001$) or the mean fluorescence intensities (data not shown) of HLA-DR⁺ monocytes measured by means of flow cytometry.

Regarding the time-course analysis, a significant increase in the whole-blood DRA mRNA level was observed in survivors (Wilcoxon's paired test, $p = .001$), whereas it remained low and stable in nonsurvivors (Fig. 5). The same trends were observed for HLA-DMA, -DMB, Ii, and CIITA but not for cathepsin S (Table 4).

DISCUSSION

The regulation of genes required for MHC II-restricted antigen presentation

plays a fundamental role in the adaptive immune response, because the encoded molecules direct the development, activation, and homeostasis of CD4⁺ T cells. During sepsis-induced immunoparalysis, the deregulated expression of monocytic HLA-DR seems to mediate a sustained depression of the antigen-presenting cell's functions (9, 10, 12). The magnitude of the immunoparalysis is believed to have a major impact in the development of secondary septic complications and thus on the patient's outcome (30, 31).

Previous studies have shown that low HLA-DR expression on monocytes from patients with sepsis may have a predictive value for a fatal outcome (11, 20, 32). It is essentially the restoration after days 4–5 of HLA-DR expression on monocytes that was found to have a good prognostic value (13, 20). In accordance, we observed a significant decreased monocytic HLA-DR expression both in survivors and nonsurvivors, with a significant recovery in patients who survived between days 1–3 and 4–10. However, we observed an increased HLA-DR expression in survivors as compared with nonsurvivors even at the early phase of the syndrome, which is in contrast with the previously published data. A monitoring study of a large cohort of patients is required to clarify this point.

Results obtained on purified monocytes from septic shock patients showed that the decreased expression of HLA-DR could be mainly mediated by reendocytosis and cellular sequestration of MHC II molecules (15). In this study, Fumeaux and Pugin observed only a slight, but not significant, down regulation of HLA-DRA mRNA production. In contrast, we found a significant correlation between the cell-surface expression of HLA-DR and the overall expression of HLA-DRB mRNA in purified monocytes, both in septic shock patients and healthy volunteers. Although our results were based on a small number of samples collected in the late phase of the syndrome, the coordinated transcriptional regulation of both HLA-DRA and -DRB chains (17) suggested that we could expect results similar to those of Fumeaux and Pugin (15). This discrepancy might be explained both by clinical and technical differences. Indeed, different monocyte purification methods, which could display different *in vitro* cell-stimulation effects and thus have a different impact on the transcriptional resumption, were used. Moreover, although

the median age of patients was similar in both studies, Fumeaux and Pugin (15) measured mRNA levels in samples collected earlier in the course of the syndrome. It is important to emphasize that Wolk and co-workers (16) demonstrated that multiple mechanisms, including a high transcriptional regulation, are involved in the reduction of HLA-DR expression in a model of lipopolysaccharide-primed monocytes. Finally, during the completion of this article, a study describing the effect of cortisol on HLA-DR down regulation reported, by using the same monocyte purification method as us, a decreased expression of HLA-DRA mRNA in a small cohort of nine

septic patients when compared with a set of control patients (33).

To get rid of the potential *in vitro* cell-stimulation effects of the monocyte purification step, we decided to extend our investigations using the PAXgene Blood RNA system. This system provides an efficient and standardized method for whole-blood collection that prevents any postsampling stimulation (34). It is important to emphasize that HLA-DR is not only expressed by circulating monocytes, which is an important limitation of using PAXgene samples. Nevertheless, in agreement with the results obtained on purified monocytes, HLA-DRB1 and -DRA mRNA levels were decreased in whole

blood from septic shock patients and showed significant correlation with the monocyte membrane HLA-DR expression. Moreover, we could not take into account the percentage of starting RNA that came from each subpopulation, which is clearly a second limitation of using the PAXgene tubes.

The genes encoding the α and β chains of all MHC II isotypes together with the genes encoding the major components of the antigen processing pathway (invariant chain and HLA-DM) are mainly regulated in a coordinated and concerted fashion by a conserved group of transcription factors (17, 29). This requires the recruitment of the MHC-specific transcriptional coactivator CIITA, which is known as the master regulator of MHC II genes (35, 36). In the present study, whole-blood mRNA levels of HLA-DRA, invariant chain, and HLA-DMA were strongly correlated together giving support to a transcriptional coregulation of these genes both in septic shock patients and healthy volunteers. As already described in multiple tissues, HLA-DRA, -DMA, and invariant chain mRNA levels were highly correlated with the CIITA mRNA expression, illustrating CIITA as the rate-limiting factor of MHC II gene expression (37). Taken together, these observations would suggest that the transcriptional regulation of CIITA is involved in the reduction of MHC II gene expression observed in the course of septic shock and is implicated in the transcriptional reinitialization observed in survivor patients.

Cathepsin S contributes to late stages of Ii degradation in antigen-presenting cells and thus allows subsequent MHC II peptide loading (38, 39). Cathepsin S transcription does not depend on CIITA regulation. In agreement, no correlation was observed between the whole-blood mRNA level of cathepsin S and CIITA or other genes required for MHC II-restricted antigen presentation. The down regulation of cathepsin S mRNA level observed in our cohort of septic shock patients and in lipopolysaccharide-primed

Table 3. Spearman's correlation coefficients between messenger RNA expression of genes required for major histocompatibility complex II-restricted antigen presentation and the transcription factor class II transactivator (CIITA) in peripheral blood

	Invariant Chain	HLA-DMA	HLA-DMB	CIITA	Cathepsin S
HLA-DRA	0.91 ^a	0.91 ^a	0.67 ^a	0.80 ^a	0.54 ^a
Invariant chain	NA	0.92 ^a	0.64 ^a	0.73 ^a	0.44 ^a
HLA-DMA	0.92 ^a	NA	0.64 ^a	0.83 ^a	0.46 ^a
HLA-DMB	0.64 ^a	0.64 ^a	NA	0.53 ^a	0.37 ^b
CIITA	0.73 ^a	0.83 ^a	0.53 ^a	NA	0.57 ^a
Cathepsin S	0.44 ^a	0.46 ^a	0.37 ^b	0.57 ^a	NA

HLA, human leukocyte antigen; NA, not applicable.

^a*p* < .0001; ^b*p* < .001.

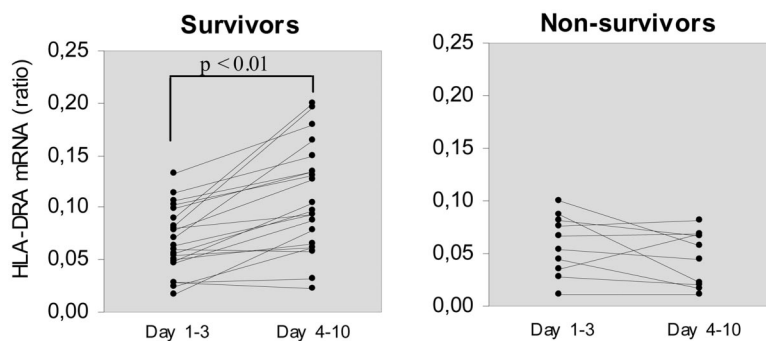


Figure 5. Time-related variation of human leukocyte antigen (HLA)-DRA messenger RNA (mRNA) level in peripheral blood. The mRNA level was measured by q-reverse transcriptase-polymerase chain reaction and normalized to that of the housekeeping gene PPIB. Individual values for survivors (n = 22) and nonsurvivors (n = 10) were taken into account when paired samples were available for the two time periods studied: days 1–3 and 4–10.

Table 4. Wilcoxon's paired test comparing the progression of messenger RNA level for the transcription factor class II transactivator (CIITA) and genes required for major histocompatibility complex II-restricted antigen presentation in septic shock patients between days 1–3 and 4–10

	DRA	CIITA	Cathepsin S	Invariant chain	DMA	DMB
Survivors (n = 22)	<i>p</i> < .01	<i>p</i> < .01	<i>p</i> = .21	<i>p</i> < .01	<i>p</i> < .01	<i>p</i> < .01
Nonsurvivors (n = 10)	<i>p</i> = .17	<i>p</i> = .066	<i>p</i> = .85	<i>p</i> = .14	<i>p</i> = .70	<i>p</i> = .44

By analyzing whole blood as well as isolated monocytes from septic shock patients, we described a global transcriptional down regulation of a panel of genes required for major histocompatibility complex class II-restricted antigen presentation.

monocytes (16) is an illustration that CIITA-independent transcriptional machinery is deactivated during immunoparalysis. The lack of significant time-course progression between days 1–3 and 4–10 observed in our study suggests that the transcriptional resumption of cathepsin S has a secondary role in the MHC II recovery observed in patients who survived.

The decreased expression of monocyte membrane MHC II observed in septic shock patients is at least partly mediated by interleukin-10 (15). However, interleukin-10-induced reduction of MHC II seems to be solely the result of reendocytosis and intracellular sequestration of mature MHC II molecules with no effect on MHC II transcription (15, 40). Transforming growth factor- β 1, catecholamine, and prostaglandin E₂ are other major antiinflammatory mediators identified in critically ill patients. Transforming growth factor- β 1 has been demonstrated *in vitro* to down regulate MHC II mRNA expression through the suppression of CIITA mRNA transcription (41, 42), whereas prostaglandin E₂ suppresses MHC II mRNA expression in macrophages (43). These observations, together with our results, suggest that the down regulation of genes required for MHC II-restricted antigen presentation observed in septic shock might be mediated by a panel of antiinflammatory mediators that remains to be described.

CONCLUSIONS

By analyzing whole blood as well as isolated monocytes from septic shock pa-

tients, we described a global transcriptional down regulation of a panel of genes required for MHC II-restricted antigen presentation. Although no direct conclusion on the mechanism taking place in the monocytes can be drawn from our PAXgene whole-blood results, we showed that the decreased surface expression of HLA-DR on monocytes was associated with the down regulation of HLA-DRA, DRB, and, to a lesser extent, with HLA-DMA and Ii mRNA levels in whole blood. This might be the result of the inhibition of various transcriptional mechanisms, of which a shutdown of CIITA mRNA expression should be crucial. In our study, although survivor and nonsurvivor patients were hardly distinguishable in the early phase of the syndrome, the time-related variation of whole-blood MHC II mRNA levels was different according to the outcome. The transcriptional resumption of CIITA-regulated genes might contribute to the recovery of membrane HLA-DR expression observed in survivor patients. These data obtained mainly from PAXgene whole-blood samples have now to be confirmed by other approaches. Defining the factors that regulate the transcription of MHC II genes will greatly contribute to a better understanding of the immunoparalysis observed in the course of sepsis.

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REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, et al: Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29:1303–1310
2. Vincent JL, Sun Q, Dubois MJ: Clinical trials of immunomodulatory therapies in severe sepsis and septic shock. *Clin Infect Dis* 2002; 34:1084–1093
3. Abraham E: Why immunomodulatory therapies have not worked in sepsis. *Intensive Care Med* 1999; 25:556–566
4. Munford RS, Pugin J: Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am J Respir Crit Care Med* 2001; 163:316–321
5. Oberholzer A, Oberholzer C, Moldawer LL: Sepsis syndromes: Understanding the role of innate and acquired immunity. *Shock* 2001; 16:83–96
6. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; 348:138–150
7. Asadullah K, Woiciechowsky C, Döcke WD, et al: Very low monocytic HLA-DR expression indicates high risk of infection—Immuno-monitoring for patients after neurosurgery and patients during high dose steroid therapy. *Eur J Emerg Med* 1995; 2:184–190
8. Muehlstedt SG, Lyte M, Rodriguez JL: Increased IL-10 production and HLA-DR suppression in the lungs of injured patients precede the development of nosocomial pneumonia. *Shock* 2002; 17:443–450
9. Manjuck J, Saha DC, Astiz M, et al: Decreased response to recall antigens is associated with depressed costimulatory receptor expression in septic critically ill patients. *J Lab Clin Med* 2000; 135:153–160
10. Wolk K, Docke WD, von BV, et al: Impaired antigen presentation by human monocytes during endotoxin tolerance. *Blood* 2000; 96: 218–223
11. Muller Kobold AC, Tulleken JE, Zijlstra JG, et al: Leukocyte activation in sepsis: Correlations with disease state and mortality. *Intensive Care Med* 2000; 26:883–892
12. Docke WD, Randow F, Syrbe U, et al: Monocyte deactivation in septic patients: Restoration by IFN-gamma treatment. *Nat Med* 1997; 3:678–681
13. Monneret G, Elmenkouri N, Bohe J, et al: Analytical requirements for measuring monocytic human lymphocyte antigen DR by flow cytometry: Application to the monitoring of patients with septic shock. *Clin Chem* 2002; 48:1589–1592
14. Kox WJ, Volk T, Kox SN, et al: Immunomodulatory therapies in sepsis. *Intensive Care Med* 2000; 26(Suppl 1):S124–S128
15. Fumeaux T, Pugin J: Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. *Am J Respir Crit Care Med* 2002; 166:1475–1482
16. Wolk K, Kunz S, Crompton NE, et al: Multiple mechanisms of reduced major histocompatibility complex class II expression in endotoxin tolerance. *J Biol Chem* 2003; 278: 18030–18036
17. Reith W, Mach B: The bare lymphocyte syndrome and the regulation of MHC expression. *Annu Rev Immunol* 2001; 19:331–373
18. Levy MM, Fink MP, Marshall JC, et al: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; 31:1250–1256
19. Le Gall JR, Lemeshow S, Saulnier F: A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993; 270:2957–2963
20. Tschaikowsky K, Hedwig-Geissing M, Schiele A, et al: Coincidence of pro- and anti-inflammatory response in the early phase of severe sepsis: Longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonine, C-reactive protein, and changes in T-cell subsets in

septic and postoperative patients. *Crit Care Med* 2002; 30:1015–1023

21. Geranton S, Rostagnat-Stefanutti A, Bendelac N, et al: High-risk genotype for type 1 diabetes: A new simple microtiter plate-based ELOSA assay. *Genet Test* 2003; 7:7–12
22. Deleted in proof
23. Deleted in proof
24. Deleted in proof
25. Pachot A, Blond JL, Mougin B, et al: Peptidylpropyl isomerase B (PPIB): A suitable reference gene for mRNA quantification in peripheral whole blood. *J Biotechnol* 2004; 114: 121–124
26. Castello R, Estelles A, Vazquez C, et al: Quantitative real-time reverse transcription-PCR assay for urokinase plasminogen activator, plasminogen activator inhibitor type 1, and tissue metalloproteinase inhibitor type 1 gene expressions in primary breast cancer. *Clin Chem* 2002; 48:1288–1295
27. Cros P, Allibert P, Mandrand B, et al: Oligonucleotide genotyping of HLA polymorphism on microtitre plates. *Lancet* 1992; 340:870–873
28. Deleted in proof
29. Boss JM, Jensen PE: Transcriptional regulation of the MHC class II antigen presentation pathway. *Curr Opin Immunol* 2003; 15:105–111
30. Majetschak M, Flach R, Kreuzfelder E, et al: The extent of traumatic damage determines a graded depression of the endotoxin responsiveness of peripheral blood mononuclear cells from patients with blunt injuries. *Crit Care Med* 1999; 27:313–318
31. Hensler T, Heidecke CD, Hecker H, et al: Increased susceptibility to postoperative sepsis in patients with impaired monocyte IL-12 production. *J Immunol* 1998; 161:2655–2659
32. Volk HD, Thieme M, Heym S, et al: Alterations in function and phenotype of monocytes from patients with septic disease—Predictive value and new therapeutic strategies. *Behring Inst Mitt* 1991; 208–215
33. Le Tulzo Y, Pangault C, Amiot L, et al: Monocyte human leukocyte antigen-DR transcriptional downregulation by cortisol during septic shock. *Am J Respir Crit Care Med* 2004; 169:1144–1151
34. Rainen L, Oelmueller U, Jurgensen S, et al: Stabilization of mRNA expression in whole blood samples. *Clin Chem* 2002; 48: 1883–1890
35. Masternak K, Muhlethaler-Mottet A, Villard J, et al: CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. *Genes Dev* 2000; 14: 1156–1166
36. Harton JA, Ting JP: Class II transactivator: Mastering the art of major histocompatibility complex expression. *Mol Cell Biol* 2000; 20: 6185–6194
37. Otten LA, Steimle V, Bontron S, et al: Quantitative control of MHC class II expression by the transactivator CIITA. *Eur J Immunol* 1998; 28:473–478
38. Riese RJ, Mitchell RN, Villadangos JA, et al: Cathepsin S activity regulates antigen presentation and immunity. *J Clin Invest* 1998; 101:2351–2363
39. Beers C, Honey K, Fink S, et al: Differential regulation of cathepsin S and cathepsin L in interferon gamma-treated macrophages. *J Exp Med* 2003; 197:169–179
40. Koppelman B, Neeffjes JJ, de Vries JE, et al: Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. *Immunity* 1997; 7:861–871
41. Czarniecki CW, Chiu HH, Wong GH, et al: Transforming growth factor-beta 1 modulates the expression of class II histocompatibility antigens on human cells. *J Immunol* 1998; 140:4217–4223
42. Lee YJ, Han Y, Lu HT, et al: TGF-beta suppresses IFN-gamma induction of class II MHC gene expression by inhibiting class II transactivator messenger RNA expression. *J Immunol* 1997; 158:2065–2075
43. Askew D, Burger CJ, Elgert KD: Tumor-induced modulation of macrophage class II MHC molecule mRNA expression. *Mol Immunol* 1993; 30:911–920