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Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: Correlation with severity and secondary septic shock

Fabienne Venet, PharmD, PhD; Sylvie Tissot, MD; Anne-Lise Debard, PharmD; Caroline Faudot; Carine Crampé; Alexandre Pachot, PharmD, PhD; Alfred Ayala, PhD; Guillaume Monneret, PharmD, PhD

Objective: Severe thermal injury causes immune dysfunctions involving both pro- and anti-inflammatory mechanisms. It subsequently leads to a state of immune deficiency that shares some similarities with sepsis-induced immunosuppression. A hallmark of the latter is established by decreased monocyte human leukocyte antigen-DR (mHLA-DR) measurements. The main objective of the current study was to characterize the appearance and the duration of low mHLA-DR expression after severe burn as well as to determine its correlation with mortality and septic complications.

Design: Observational study.

Setting: Burn unit (intensive care unit) in a university hospital.

Patients: Severe burn patients (total burn surface area >30%, n = 14) and healthy individuals (n = 29).

Interventions: None.

Measurements and Main Results: Patients were immunologically monitored during 15 days. We quantified mHLA-DR expression with a standardized flow cytometry protocol. Every patient presented with decreased mHLA-DR expression at days 2–3 after

burn. Then, from days 4–6, this expression increased in patients who would survive whereas it remained low in nonsurvivors. As early as days 7–10 after burn, patients who were going to develop secondary septic shock exhibited significantly lower mHLA-DR expression in comparison with patients recovering without severe septic complications. Using quantitative reverse transcriptase-polymerase chain reaction, at days 4–6, we found that the RNA level of the nonpolymorphic HLA-DRA chain and the transcription factor class II transactivator were also significantly decreased compared with healthy controls; however, plasma cytokines (interleukin-6, tumor necrosis factor- α , and interleukin-10) did not provide any significant prognostic information.

Conclusions: Severe burn injury induced a marked reduction in mHLA-DR expression at both protein and messenger RNA levels. Its persistent decrease was associated with mortality and the development of septic complications. (Crit Care Med 2007; 35:1910–1917)

KEY WORDS: severe burn; monocyte; human leukocyte antigen-DR; immunosuppression; septic shock

Burns remain a major cause of injury and death worldwide (1, 2). Increasing patient age, increasing burn size, and inhalation injury are factors that increase the risk of death after major burn injury. Secondary infection is also a leading factor of morbidity and mortality. After the initial resuscitation, 70% to 75% of mortality is related to infection (1, 3).

It has been postulated for a long time that severe thermal injuries may provoke a state of immunosuppression (4). Burn injury induces the activation of a proin-

flammatory response that is thought to be followed by a subsequent anti-inflammatory phase, in an effort to maintain inflammatory immune homeostasis. However, this compensatory feedback may also contribute to a state of immunosuppression (4). Among other depressed functions, monocyte alterations have been suggested to play a critical role in the development of burn-induced immunodepression (1). It has been proposed that these alterations in the innate immune system exhibit some similarities with the state of “immunoparalysis” or

“leukocyte reprogramming” observed after septic shock, which is thought to decrease the natural defenses that are responsible for counteracting pathogens. As a result, burn patients would be more susceptible to wound infections, severe sepsis, and subsequent multiple organ failure (1). A diagnostic laboratory marker would therefore be desirable to help in identifying immunosuppressed patients at high risk for infectious complications after severe burn.

Decreased monocyte human leukocyte antigen-DR (mHLA-DR) expression has been demonstrated to be a reliable marker for monitoring immunosuppression in critically ill patients. Strongly diminished mHLA-DR expression has been used to identify patients with temporary immunosuppression who will present with secondary nosocomial infections after various injuries: trauma (5, 6), acute pancreatitis (7), cardiac surgery (8), transplantation (9), and severe sepsis (10). Decreased HLA-DR expression has also been shown to be an independent

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predictor of mortality in septic shock (11). However, it is unclear whether these findings extend to burn patients.

The aim of the present study was thus to assess mHLA-DR expression in burn patients. Plasma cytokine measurements of interleukin (IL)-6, tumor necrosis factor (TNF)- α , and IL-10 were also determined. Subsequently, we determined whether these variables could be associated with mortality and with the development of severe septic complications.

PATIENTS AND METHODS

Patients. Burn victims admitted to the burn unit at E. Herriot University Hospital were prospectively included in the study. Exclusion criteria included all patients younger than 18 yrs and with total burn surface area (TBSA) injury <30%. Mortality was defined as death occurring within burn unit stay. The institutional ethics committee reviewed and approved this observational study.

Diagnosis of Secondary Infections. The diagnosis of infection and septic shock was based on criteria of the American College of Chest Physicians/Society of Critical Care Medicine (12). As surface swabs and cultures cannot distinguish infection from colonization (3), wound infections are not taken into account in our routine follow-up, except in case of graft failure or when associated with a least one systemic sign of infection. Septic shock was defined by an identifiable site of infection and evidence of a systemic inflammatory response manifested by at least two of the following criteria: a) a body temperature >38°C or <36°C; b) heart rate >90 beats/min; c) respiratory rate >20 breaths/min; d) white blood cell count >12,000/mm³ or <4000/mm³; and e) blood pressure \leq 90 mm Hg despite fluid resuscitation and requiring vasopressor therapy (>5 μ g/kg of body weight of epinephrine and/or norepinephrine and/or dobutamine).

Samples. EDTA-anticoagulated samples were drawn in the following time period after thermal injury: day 1 (first 24 hrs), days 2–3, days 4–6, days 7–10, and days 11–15 (one sample per period).

Plasma Cytokine Measurements. Plasma levels of IL-10, TNF- α , and IL-6 were measured using commercial enzyme-linked immunosorbent assay kits, according to the manufacturers' recommendations (Biosource, Camarillo, CA, for IL-10 and TNF- α ; Immunotech, Marseille, France, for IL-6). The detection limits of the assays (and normal values given by manufacturers) were 1 pg/mL (<10 pg/mL) for IL-10, 3 pg/mL (<15 pg/mL) for TNF- α , and 3 pg/mL (<7 pg/mL) for IL-6.

Monocyte HLA-DR Measurement by Flow Cytometry. Flow cytometric (FC500, Beckman-Coulter, Hialeah, FL) expression of mHLA-DR was assessed on peripheral blood. Staining and cell acquisition on flow cytometer were per-

formed within 2 hrs after blood sampling (storage at 4°C). Monoclonal antibodies were used according to the manufacturers' recommendations: fluorescein isothiocyanate-labeled anti-CD14 (10 μ L, Immunotech, Marseille, France) and PE-labeled anti-HLA-DR or PE-labeled corresponding isotype control (20 μ L, Becton Dickinson-Pharmingen, San Jose, CA) per 100 μ L of whole blood. As previously described (11), monocytes (1500 from each sample) were characterized on the basis of their CD14 expression. Results were expressed either as percentages of HLA-DR-positive monocytes of total monocyte population (the threshold for positivity was defined with the isotype control and settled on 2% of positive cells) or as a number of PE-conjugated antibodies bound per cell (AB/C) that was determined with BD Quantibrite PE beads (Becton Dickinson) as previously standardized in a multicenter study (13).

HLA-DRA and CIITA Messenger RNA Quantification in Whole Blood Samples. Blood samples were collected directly in PAX-Gene Blood RNA tubes (PreAnalytix, Hilden, Germany), and 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 using the ThermoScript reverse transcriptase-polymerase chain reaction system (Invitrogen) according to the manufacturer's instructions. The messenger RNA (mRNA) expression of HLA-DRA and the transcription factor class II transactivator (CIITA) was quantified by reverse transcriptase-polymerase chain reaction using the LightCycler instrument and the Fast-Start DNA Master SYBR Green I real-time PCR kit (Roche Molecular Biochemicals, Basel, Switzerland) as previously described (14). HLA-DRA and CIITA mRNA level were normalized to that of the housekeeping gene peptidylpropyl isomerase B encoding for cyclophilin B as previously described (15). The complementary DNA calibrators for HLA-DRA and CIITA were prepared from purified polymerase chain reaction amplicons obtained with the primers described previously (15). Standards and ready-to-use primer mixes for peptidylpropyl isomerase B were obtained from Search-LC (Heidelberg, Germany). Using the LightCycler Software version 4, the results were expressed as a concentration ratio of mRNA/peptidylpropyl isomerase B mRNA. Twenty-nine patients from hospital staff served as control values for mRNA measurements.

Statistical Analysis. Median values are reported together with interquartile ranges. Comparisons between groups were made using a nonparametric Mann-Whitney U test for continuous variables and the chi-square test for categorical data. A *p* value <.05 was regarded as significant without correction of the number of tests performed.

RESULTS

Patients. Samples were obtained from 14 burn patients (five females, nine males; median age = 44.5 yrs, range 18–74 yrs). The baseline characteristics of those patients are presented in Table 1. The TBSA ranged from 32% to 85% (median 50%). Three patients presented with additional inhalation injury. Initial resuscitation was carried out on the basis of the Baxter's Parkland Hospital formula (16). Early enteral feeding was established during initial resuscitation and progressively increased to reach a caloric intake of 35 kcal/kg/day. Silver sulfadiazine was used as topical antimicrobial. Surgical treatment, including excision and skin grafting, was usually started in the first week (median day 6, range day 3 to day 11) and then continued with a frequency of once or twice a week. Four patients died (mortality 28.6%). Before day 15, all but two patients (one survivor and one nonsurvivor) developed secondary infections: bacteremia (*n* = 3, *Staphylococcus aureus*, *Escherichia coli*) or pneumonia (*n* = 11, *S. aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Haemophilus influenzae*, *E. coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*). In this cohort of patients, we did not identify invasive wound infections or catheter-related infections. Septic shock was diagnosed in five patients between day 16 and day 28 after burn (incidence 36%). The bacterial species involved were *P. aeruginosa* (three cases), *Bacteroides* species (one case), and *P. mirabilis* (one case). Among those patients, three died. The fourth nonsurviving patient died before day 15 after burn due to multiple organ failure without any septic complications. Nonsurviving patients presented with a significantly higher Abbreviated Burn Severity Index score than survivors (Table 2). Patients presenting with delayed septic shock received significantly more red blood cell units and presented significantly more inhalation injury than nonseptic patients (Table 2). We did not observe any difference in TBSA, time of first surgery, number of surgery procedures, or number of infections before day 15 between these different groups of patients (Table 2).

Plasma Cytokine Concentrations. In the whole population, median values (and interquartile ranges) for sampling days were as follows: 2 (2–2), 4 (4–5), 9 (7–9), and 12 (12–14) corresponding to day 2–3, day 4–6, day 7–10, and day 11–15 periods

of monitoring, respectively. There was no difference in sampling times regarding the different groups of patients. IL-6 concentrations were elevated immediately after

burn but then declined over the duration of the whole monitoring period (Table 3). Values tended to be higher in nonsurviving patients, although this was

not statistically significant (Table 3). Alternatively, circulating TNF- α concentrations remained increased during the whole monitoring phase. Nonsurviving patients tended to exhibit higher TNF- α concentrations than survivors (Table 3). When considering maximal TNF- α value, it was significantly higher in nonsurviving vs. surviving patients (54 pg/mL vs. 29 pg/mL, $p = .034$). Surprisingly, IL-10 concentrations were generally below the detection limit of the kit, except at day 1 (Table 3) and provided no prognostic information.

mHLA-DR Expression in Whole Patient Population. Results are presented either as percentages of monocytes expressing HLA-DR (normal >90%) or as number of anti-HLA-DR AB/C (normal >15,000), which is correlated with the number of HLA-DR molecules expressed on each monocyte (13). Burn trauma caused a pronounced decrease of

Table 1. Demographic and clinical characteristics of the 14 severe burn patients included in the study

Patient	Age, yrs	Gender	TBSA, %	ABSI Score	Inhalation	Length of Stay, Days	Survival	Septic Shock
1	22	M	32	7	No	29	S	No
2	18	M	60	9	No	32	S	No
3	56	F	80	15	Yes	38	NS	Yes
4	56	F	48	10	Yes	22	NS	Yes
5	34	M	50	9	No	68	S	No
6	47	M	51	13	No	91	S	No
7	45	F	80	10	No	27	NS	Yes
8	40	F	50	9	No	45	S	Yes
9	74	M	41	10	No	24	S	No
10	51	M	45	9	No	25	S	No
11	27	M	45	8	No	21	S	No
12	44	F	50	9	Yes	66	S	Yes
13	51	M	85	12	No	11	NS	—
14	40	M	85	12	No	17	S	No

TBSA, total burn surface area; ABSI, Abbreviated Burn Severity Index; S, survivor; NS, nonsurvivor.

Table 2. Demographic and clinical characteristics of the 14 severe burn patients regarding issue and development of septic shock between day 15 and day 28 after burn

	Age, yrs	Gender, Male/Female	TBSA, %	ABSI Score	RBC, Units	Inhalation, Yes/No	Time of First Surgery, Days	No. of Surgical Procedures	No. of Infections Before Day 15	Length of Stay, Days
Survivors (n = 10)	40 (29–46)	8/2	50 (45–51)	9 (9–9.75)	16 (10–37)	1/9	6 (4–8)	3.5 (3–4)	1 (1–1)	31 (24–61)
Nonsurvivors (n = 4)	53.5 (50–56)	1/3	80 (72–81)	11 (10–13) ^a	30 (20–44)	2/2	5.5 (3.5–7)	4 (2.5–4)	1 (0.5–1.5)	24.5 (19–30)
Septic shock after day 15										
No (n = 8)	37 (26–48)	8/0	47.5 (44–53)	10 (9–10)	12.5 (10–19)	0/8	7.5 (4.5–9)	3 (2.5–4)	1 (1–1)	27 (23–41)
Yes (n = 5)	45 (44–56)	0/5 ^b	50 (50–80)	9 (9–11)	57 (36–68) ^b	3/2 ^a	4 (3.75–5.5)	4 (4–4)	1 (1–2)	38 (27–45)

TBSA, total burn surface area; ABSI, Abbreviated Burn Severity Index; RBC, red blood cell.

^a $p < .05$; ^b $p < .01$. Median and interquartile ranges are presented (Q1–Q3). Comparisons between groups were made using a nonparametric Mann-Whitney U test for continuous variable and the chi-square test for categorical data.

Table 3. Time course of cytokine measurements in 14 severe burn patients

	Days After Burn				
	0–1	2–3	4–6	7–10	11–15
IL-6 (normal <7)					
Survivors	766 (624–1183)	565 (440–713)	332 (231–405)	173 (128–339)	154 (107–227)
Nonsurvivors	1039 (613–1145)	661 (388–5273)	387 (288–561)	248 (74–861)	287 (160–959)
Without septic shock	708 (596–1302)	565 (445–738)	332 (262–442)	173 (129–289)	113 (99–186)
Septic shock >day 15	932 (665–1092)	388 (99–673)	288 (188–391)	116 (76–420)	287 (252–369)
TNF- α (normal <15)					
Survivors	17 (12–17)	19 (14–21)	18 (14–23)	23 (21–38)	30 (26–33)
Nonsurvivors	36 (27–45)	29 (27–36)	40 (35–41)	46 (40–52)	39 (34–51)
Without septic shock	16 (10–17)	19 (15–22)	21 (17–23)	23 (21–45)	32 (28–36)
Septic shock >day 15	27 (18–41)	23 (19–26)	22 (14–33)	32 (23–42)	29 (27–39)
IL-10 (normal <10)					
Survivors	13 (6–19)	<5 (<5–5)	<5 (<5–5)	<5 (<5–7)	<5 (<5–<5)
Nonsurvivors	<5 (<5–236)	<5 (<5–6)	7 (6–8)	9 (<5–24)	<5 (<5–15)
Without septic shock	7 (6–19)	<5 (<5–5)	<5 (<5–6)	<5 (<5–8)	<5 (<5–<5)
Septic shock >day 15	12 (<5–131)	<5 (<5–5)	<5 (<5–7)	<5 (<5–<5)	<5 (<5–<5)

IL, interleukin; TNF, tumor necrosis factor.

Data are expressed as median and interquartile ranges (Q1–Q3) of cytokine concentrations in pg/mL.

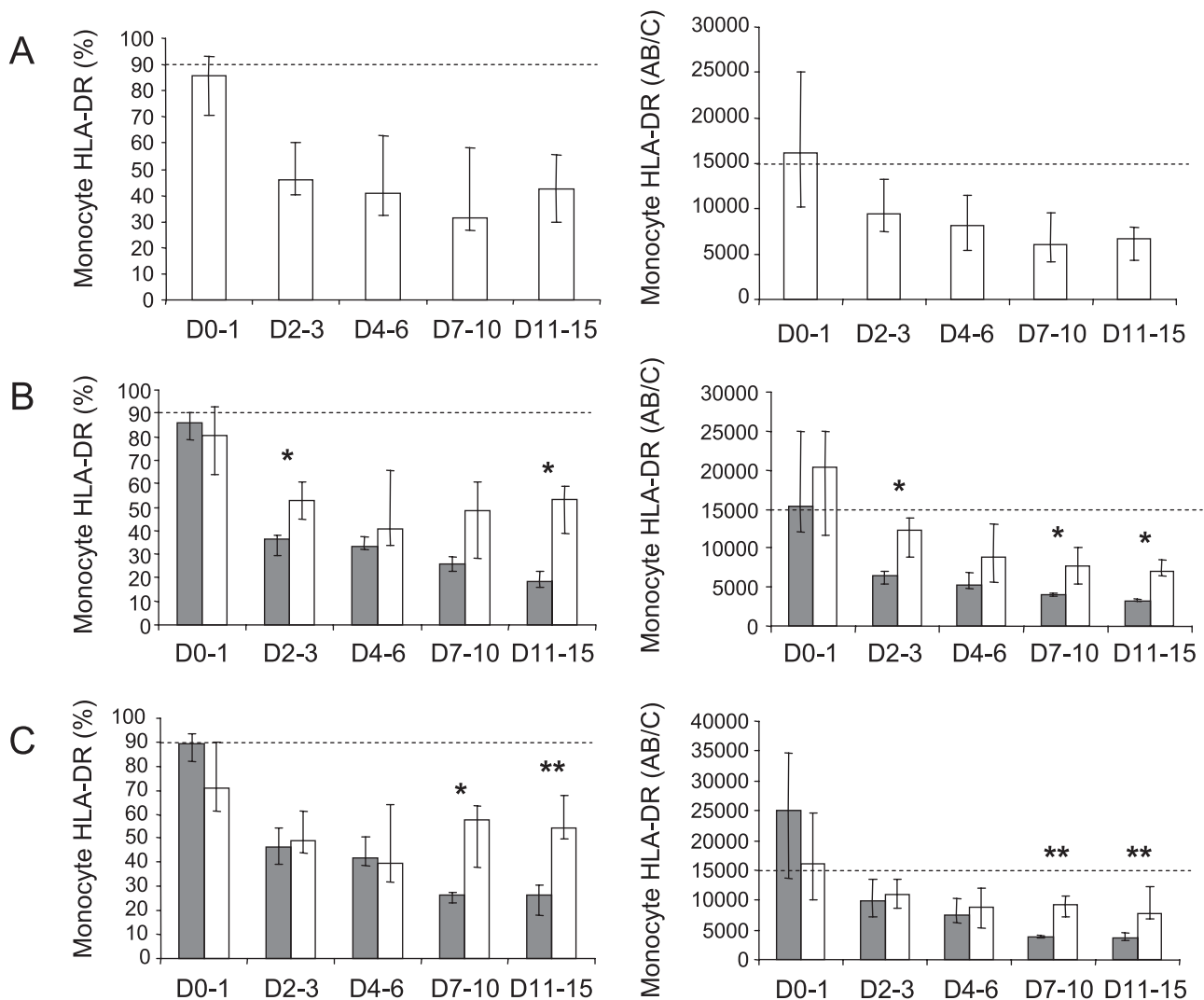


Figure 1. Time course of monocyte human leukocyte antigen-DR (HLA-DR) expression in severe burn patients. Median and interquartile ranges (Q1–Q3) are presented. Results are expressed as percentages of monocyte expressing HLA-DR (*left panels*) and as numbers of anti-HLA-DR antibodies bound per cell (AB/C) (*right panels*). The nonparametric Mann-Whitney U test was used for comparison between groups. * $p < .05$; ** $p < .01$. *A*, monocyte HLA-DR expression in the whole burn population ($n = 14$ severe burn patients). *B*, monocyte HLA-DR expression regarding the outcome ($n = 10$ surviving patients, *open columns*, and $n = 4$ nonsurvivors, *filled columns*). *C*, monocyte HLA-DR expression regarding the development of septic shock between day (D) 15 and day 28 after burn injury ($n = 8$ nonseptic patients, *open columns*, and $n = 5$ septic patients, *filled columns*). Dashed lines represent healthy individual values.

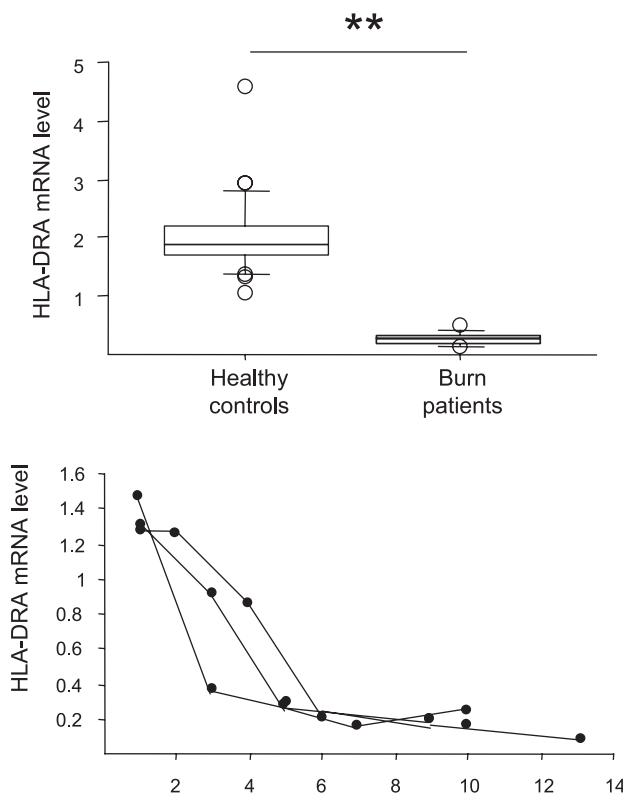
mHLA-DR expression. Although at days 0–1 the percentages of monocytes expressing HLA-DR were not different from healthy individual values (median 86%, Q1–Q3 70.9–93.2), as little as 2–3 days after burn, patients exhibited a major decrease of this percentage, and this was sustained through days 4–6 or 7–10 (Fig. 1A). Similar results were observed when HLA-DR expression was presented as AB/C (Fig. 1A). When HLA-DRA and CIITA mRNA expressions were measured at days 4–6 (a time period when mHLA-DR expression was already diminished in all patients), we found a significant downmodulation of mRNA in whole blood (Fig. 2). This was similar to what we had previously observed in septic shock (14), in-

dicating a transcriptional regulation of genes involved in HLA-DR expression. Daily variations in mRNA levels showed a similar profile to that of cell-surface mHLA-DR expression: a rapid decrease in the first days followed by a plateau at lower values (Fig. 2).

mHLA-DR Expression According to Mortality and Secondary Septic Shock. After the first day, we observed that non-surviving patients exhibited lower mHLA-DR values than survivors during the residual monitoring period (Fig. 1B), with a statistically significant difference reached at days 2–3 (36% vs. 53%, $p = .028$) and at days 11–15 (26% vs. 48%, $p = .013$). Similar results were observed when data were expressed as AB/C, with a

statistically significant difference observed at days 2–3 (6500 vs. 12,300, $p = .028$), days 7–10 (4000 vs. 7700, $p = .034$), and days 11–15 (3200 vs. 7000, $p = .013$). At the two time points just preceding the diagnosis of septic shock, patients with secondary septic shock presented with a significantly lower percentage of HLA-DR-positive monocytes: 26% vs. 58% ($p = .013$) and 27% vs. 55% ($p = .007$) at days 7–10 and days 11–15, respectively (Fig. 1C). We found comparable AB/C results: 3900 vs. 9100 ($p = .003$) and 3500 vs. 7800 ($p = .005$) at days 7–10 and days 11–15, respectively (Fig. 1C). The nadir of mHLA-DR expression occurred at days 7–10 after burn. Significantly lower nadir values were observed in nonsurviving vs. surviving pa-

A / HLA-DRA



B / CIITA

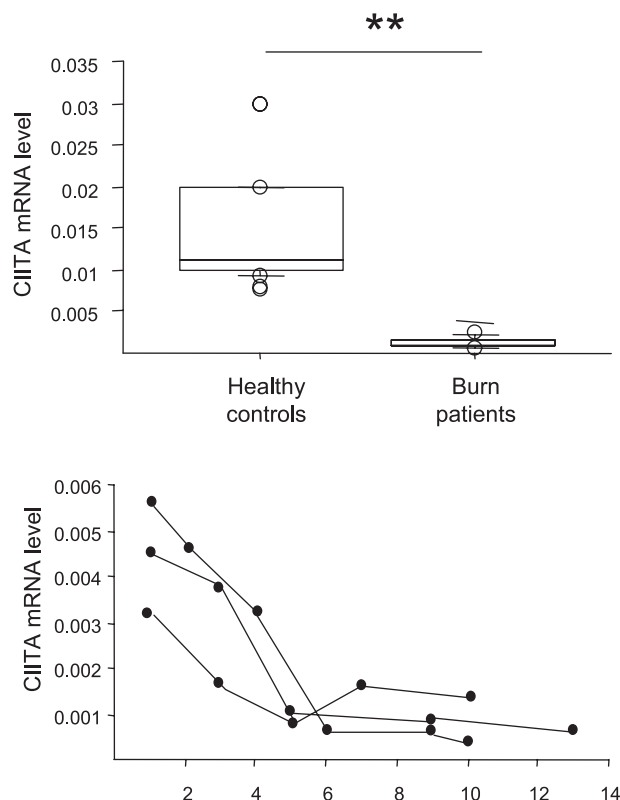


Figure 2. Messenger RNA (mRNA) level of monocyte human leukocyte antigen-DR (HLA-DRA) (A) and class II transactivator (CIITA) (B) in peripheral blood of severe burn patients measured by quantitative reverse transcription-polymerase chain reaction. The mRNA level was normalized to that of the housekeeping gene peptidylprolyl isomerase B. The upper box plot represents mRNA levels in patients (n = 9) at days 4–6 after burn injury and healthy controls (n = 29). The Mann-Whitney nonparametric U test was used for comparison between groups. ** $p < .01$. The lower kinetics represent time-related individual variation of mRNA level in three burn patients, representative of the whole cohort.

tients (Fig. 3, A-a and B-a) and in secondary septic shock patients vs. nonseptic patients (Fig. 3, A-b and B-b). Moreover, reduced nadir values were observed in patients exhibiting inhalation injury (Fig. 3, A-c and B-c) and increased Abbreviated Burn Severity Index score (Fig. 3, A-d and B-d). Finally, the mHLA-DR nadir occurred later in nonsurvivors or in patients who developed septic shock. It occurred at days 11–15 in nonsurvivors vs. days 4–6 in survivors ($p = .17$) and similarly at days 11–15 in septic patients vs. days 4–6 in nonseptic ones ($p = .01$).

DISCUSSION

Our findings show that severe thermal injury induced a marked decrease in mHLA-DR expression at both the protein and mRNA levels. Its persistence and intensity were associated with pejorative outcome and with the development of secondary septic shock.

Severe burn represents a frequent trauma in industrial countries and a ma-

jor healthcare problem in developing countries (1). Mortality of severe burn patients has decreased substantially in the past few decades in specialized centers, due in large part to progress in the provision of optimized fluid resuscitation, nutritional support, and pulmonary care (1). However, despite these advances, severe sepsis and subsequent multiple organ failure remain a major source of burn morbidity and mortality. In fact, in patients with severe burns, 75% of all deaths are related to secondary sepsis (1).

Initially, the immune response after thermal injury is dominated by the proinflammatory response but later becomes predominantly anti-inflammatory in nature. This latter condition is thought to lead to a state of immunosuppression (1). These alterations of the immune system may participate in increasing patients' susceptibility to infectious complications. Their development, however, is complex and likely involves multiple factors and

processes that remain to be fully elucidated, including any deleterious effect of patients' treatments. For example, it is speculated that early excision may be protective although requiring more blood transfusions (17). In contrast, it has been also proposed that the number of blood transfusions may be associated with infectious complications (18). Accordingly, we observed that patients who developed septic shock received more red blood cell units than nonseptic patients. However, to clearly delineate a relationship between septic onset and the different treatment strategies, further investigations and multivariate analysis in larger cohorts of patients are needed.

Regarding cytokines, consistent with previous observations (19, 20), we found an initial elevation in IL-6 concentrations, which declined thereafter. Circulating TNF- α levels were also moderately increased in burn patients when compared with normal values. In contrast, IL-10 concentrations were generally

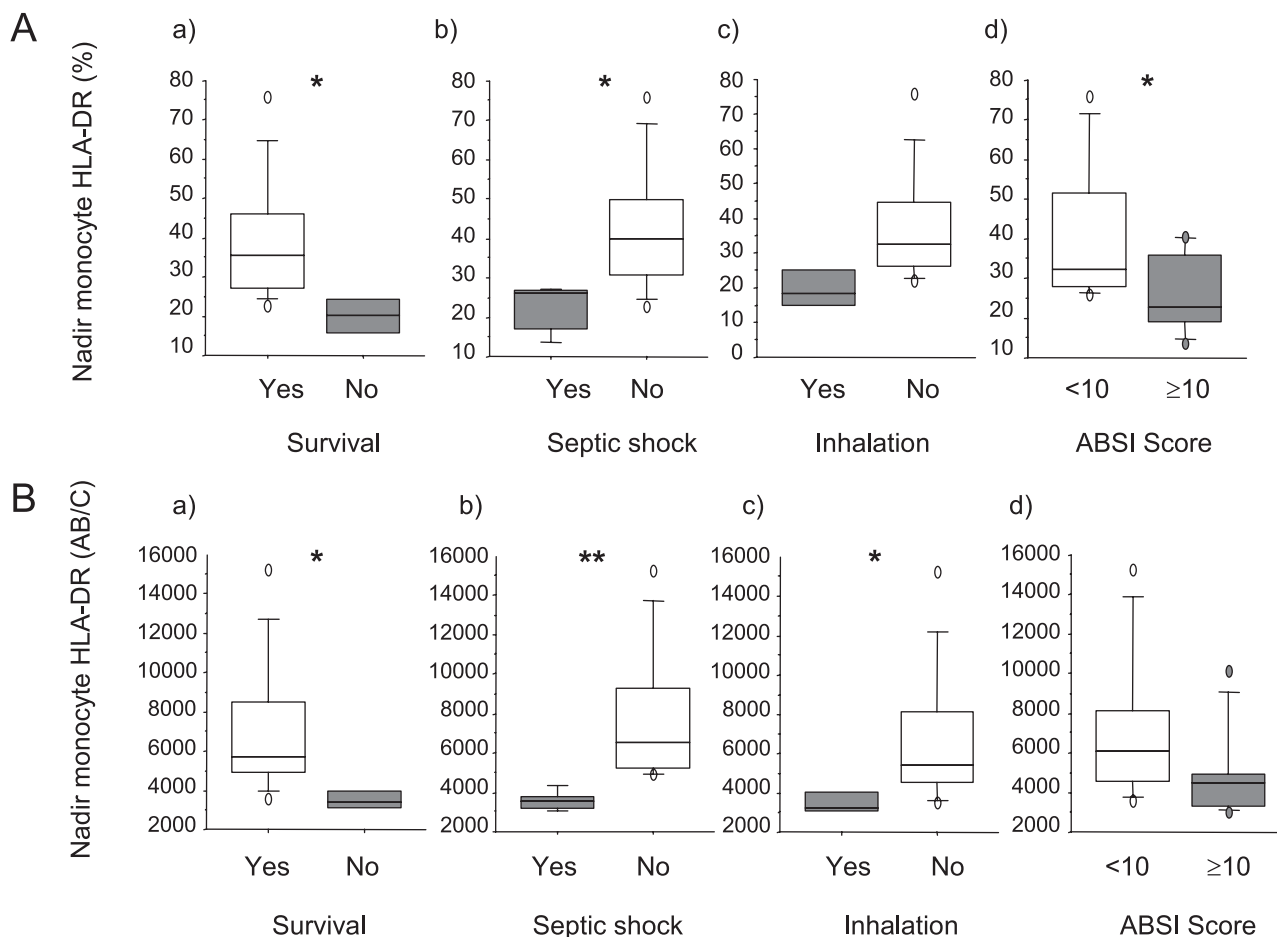


Figure 3. Box plot representations of the nadir of monocyte human leukocyte antigen-DR (HLA-DR) expression in severe burn patients. Results are expressed as percentages of monocyte expressing HLA-DR (A) and as numbers of anti-HLA-DR antibodies bound per cell (AB/C) (B). The Mann-Whitney nonparametric U test was used for comparison between groups. * $p < .05$; ** $p < .01$. a, nadir of monocyte HLA-DR expression in surviving (open box plot, $n = 10$) and nonsurviving (filled box plot, $n = 4$) burn patients. b, nadir of monocyte HLA-DR expression in burn patients developing a septic shock (filled box plot, $n = 5$) and nonseptic patients (open box plot, $n = 8$). c, nadir of monocyte HLA-DR expression regarding the occurrence of an initial inhalation injury in severe burn patients. Filled box plot, patients with inhalation injury ($n = 3$); open box plot, patients without inhalation injury ($n = 11$). d, nadir of monocyte HLA-DR expression regarding Abbreviated Burn Severity Index score (ABSI). Open box plot, patients with an ABSI score <10 ($n = 7$); filled box plot, patients with ABSI score ≥ 10 ($n = 7$).

found to be below the detection limit of the assay. The putative involvement of IL-10 in postburn immunosuppression remains unclear (21, 22), and the present data do not support a role for this immunosuppressive cytokine. Taken together, these results suggest, as already proposed, that the occurrence of an initial proinflammatory response takes place rapidly after the initial burn injury (23). However, we did not observe any correlation between cytokine concentrations and the development of severe septic complications, unfavorable outcome, other clinical confounders, or mHLA-DR measurements.

The diminished mHLA-DR expression is proposed to be a reflection of the immunosuppressive state in critically ill patients. Indeed, it is known that monocytes from patients with low mHLA-DR

are unable to produce TNF and IL-1 upon an additional bacterial challenge (24) or to induce T-lymphocyte proliferation in response to recall antigen (25). *Ex vivo* experiments have shown that as mHLA-DR recovers, this is accompanied by the restoration of TNF release capacity (with granulocyte macrophage colony-stimulating factor) (26) and increased T proliferation and proinflammatory cytokine production (with granulocyte colony-stimulating factor) (27). In clinical studies, diminished mHLA-DR expression has been correlated with septic complications after various injuries: trauma, surgery, or pancreatitis (5–7, 28). In children undergoing cardiac surgery, low mHLA-DR has also been identified as an independent predictor of septic complications after adjustment for clinical variables (8). In septic shock patients, re-

duced mHLA-DR expression has been recently demonstrated to be an independent predictor of mortality in multivariate analysis, including various clinical confounders (11). Only fragmented data are available regarding mHLA-DR expression after severe burn, and these were not collected using a standardized protocol (22, 29, 30). However, these data tended to indicate that low mHLA-DR expression was associated with secondary septic events. In the present study, we examined mHLA-DR expression in severe burn patients according to these recently standardized protocols (11, 13), and we confirmed our observations at the mRNA level. While proinflammatory cytokine response rapidly developed after burn, mHLA-DR expression initially was not different from normal values. Then, we observed as early as 2–3 days after ther-

mal injury a severe decrease of mHLA-DR expression. This loss of mHLA-DR expression is suggestive of a counterregulatory response. It might be thought of as a consequence of homeostatic maintenance aimed at controlling the inflammatory balance. However, this reduction in monocyte HLA-DR expression was not accompanied by increased circulating IL-10 concentrations, even though this cytokine has been found to be associated with low mHLA-DR in severe sepsis/septic shock (31, 32). This suggests that while a similar process (an mHLA-DR loss) occurs in both situations, a different mechanism may sustain the observed suppression after thermal injury. As an aside, we have also noted that the kinetics of mHLA-DR expression seem to be different in burn patients as opposed to septic shock patients. In septic shock (11), the nadir mHLA-DR value occurred in the first 2 days, whereas in the study of thermal injury, the nadir mHLA-DR value was observed at days 7–10. Here again, while this implies that processes underlying these changes in burn differ from septic shock, specific reasons for these differences remain to be investigated.

As observed after septic shock (10, 11), the decreased monocyte HLA-DR expression provided prognostic information. It was more pronounced and persistent in nonsurviving patients and in patients developing a secondary septic shock. From days 4 to 6, mHLA-DR values increased in nonseptic patients whereas they remained very low in patients with severe septic complications. Most important, at days 7–10 and 11–15, there were significant differences between patients without septic shock and those who went on to present with septic shock after day 16. Last, lower mHLA-DR values were observed in patients with increased Abbreviated Burn Severity Index score and with inhalation injury, which constitute well-known risk factors after burn (33). The severity of the initial burn insult may thus be associated with the intensity of the subsequent immunosuppressive state. All these putative correlations need to be further investigated in multicenter studies including large numbers of patients. For this purpose, a standardized assay for the measurement of mHLA-DR expression is now validated, and preliminary cutoff levels have been proposed: a value <5000 AB/C identifies patients with immunosuppression and enhanced risk of developing infectious complications (13). In previous studies, a threshold of

30% of HLA-DR positive monocytes was regularly found to differentiate survivors from nonsurvivors after severe sepsis/septic shock (11, 31, 34). Accordingly, in the present study, using either 30% or 5000 AB/C for cutoff values, nonsurviving patients and patients developing secondary septic shock could be rapidly identified. In the other patients, mHLA-DR expression never declined under these thresholds.

CONCLUSIONS

We observed that after severe thermal injury, HLA-DR expression is severely decreased on circulating monocytes and that the duration and the intensity of this decrease are associated with severe septic complications and pejorative outcome. In this situation, cytokine measurements did not provide any prognostic information. After confirmation of these preliminary observational results in larger cohorts of patients (including functional testing, such as TNF intracellular staining or *in vitro* TNF release after stimulation), mHLA-DR expression measurement could be used as a monitoring tool to detect as early as possible immunocompromised patients who might benefit from interventional therapies that could reduce the risk of developing severe secondary septic events.

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REFERENCES

- Church D, Elsayed S, Reid O, et al: Burn wound infections. *Clin Microbiol Rev* 2006; 19:403–434
- Hettiaratchy S, Dziewulski P: ABC of burns. Introduction. *BMJ* 2004; 328:1366–1368
- Ansermino M, Hemsley C: Intensive care management and control of infection. *BMJ* 2004; 329:220–223
- Schwacha MG: Macrophages and post-burn immune dysfunction. *Burns* 2003; 29:1–14
- Ditschkowski M, Kreuzfelder E, Rebmann V, et al: HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. *Ann Surg* 1999; 229:246–254
- 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

- Satoh A, Miura T, Satoh K, et al: Human leukocyte antigen-DR expression on peripheral monocytes as a predictive marker of sepsis during acute pancreatitis. *Pancreas* 2002; 25:245–250
- Allen ML, Peters MJ, Goldman A, et al: Early postoperative monocyte deactivation predicts systemic inflammation and prolonged stay in paediatric cardiac intensive care. *Crit Care Med* 2002; 30:1140–1145
- Haveman JW, van den Berg AP, van den Berk JM, et al: Low HLA-DR expression on peripheral blood monocytes predicts bacterial sepsis after liver transplantation: Relation with prednisolone intake. *Transpl Infect Dis* 1999; 1:146–152
- 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
- Monneret G, Lepape A, Voirin N, et al: Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intensive Care Med* 2006; 32: 1175–1183
- Bone RC, Balk RA, Cerra FB, et al: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101:1644–1655
- Docke WD, Hoflich C, Davis KA, et al: Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: A multicenter standardized study. *Clin Chem* 2005; 51:2341–2347
- Pachot A, Monneret G, Brion A, et al: Messenger RNA expression of major histocompatibility complex class II genes in whole blood from septic shock patients. *Crit Care Med* 2005; 33:31–38
- 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
- MacLennan N, Heimbach DM, Cullen BF: Anesthesia for major thermal injury. *Anesthesiology* 1998; 89:749–770
- Ong YS, Samuel M, Song C: Meta-analysis of early excision of burns. *Burns* 2006; 32: 145–150
- Palmieri TL, Caruso DM, Foster KN, et al: Effect of blood transfusion on outcome after major burn injury: A multicenter study. *Crit Care Med* 2006; 34:1602–1607
- Biffi WL, Moore EE, Moore FA, et al: Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? *Ann Surg* 1996; 224:647–664
- Yeh FL, Lin WL, Shen HD, et al: Changes in circulating levels of interleukin 6 in burned patients. *Burns* 1999; 25:131–136
- Yeh FL, Lin WL, Shen HD: Changes in circulating levels of an anti-inflammatory cyto-

- kine interleukin 10 in burned patients. *Burns* 2000; 26:454–459
22. Sachse C, Prigge M, Cramer G, et al: Association between reduced human leukocyte antigen (HLA)-DR expression on blood monocytes and increased plasma level of interleukin-10 in patients with severe burns. *Clin Chem Lab Med* 1999; 37:193–198
 23. Schwacha MG, Chaudry IH: The cellular basis of post-burn immunosuppression: Macrophages and mediators. *Int J Mol Med* 2002; 10:239–243
 24. Astiz M, Saha D, Lustbader D, et al: Monocyte response to bacterial toxins, expression of cell surface receptors, and release of anti-inflammatory cytokines during sepsis. *J Lab Clin Med* 1996; 128:594–600
 25. 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
 26. Nierhaus A, Montag B, Timmler N, et al: Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis. *Intensive Care Med* 2003; 29:646–651
 27. Schneider C, von Aulock S, Zedler S, et al: Perioperative recombinant human granulocyte colony-stimulating factor (Filgrastim) treatment prevents immunoinflammatory dysfunction associated with major surgery. *Ann Surg* 2004; 239:75–81
 28. Hershman MJ, Cheadle WG, Wellhausen SR, et al: Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. *Br J Surg* 1990; 77:204–207
 29. Gibbons RA, Martinez OM, Lim RC, et al: Reduction in HLA-DR, HLA-DQ and HLA-DP expression by Leu-M3+ cells from the peripheral blood of patients with thermal injury. *Clin Exp Immunol* 1989; 75:371–375
 30. Zwadlo-Klarwasser G, Kauhl W, Schmitz C, et al: Influence of severe burn injury on the expression of RM 3/1 and HLA-DR antigens in human blood monocytes. *J Burn Care Rehabil* 1996; 17:287–293
 31. Lekkou A, Karakantza M, Mouzaki A, et al: Cytokine production and monocyte HLA-DR expression as predictors of outcome for patients with community-acquired severe infections. *Clin Diagn Lab Immunol* 2004; 11:161–167
 32. Monneret G, Finck ME, Venet F, et al: The anti-inflammatory response dominates after septic shock: Association of low monocyte HLA-DR expression and high interleukin-10 concentration. *Immunol Lett* 2004; 95: 193–198
 33. Fitzwater J, Purdue GF, Hunt JL, et al: The risk factors and time course of sepsis and organ dysfunction after burn trauma. *J Trauma* 2003; 54:959–966
 34. Docke WD, Randow F, Syrbe U, et al: Monocyte deactivation in septic patients: Restoration by IFN-gamma treatment. *Nat Med* 1997; 3:678–681