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Scientific Letter

Monocyte HLA-DR Expression to Monitor Immune Response and Potential Infection Risks Following Vaso-Occlusive Crises in Patients with Sickle Cell Anemia

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To the editor.

Sickle cell anemia (SCA) is a genetic disorder characterized by chronic hemolysis and vascular dysfunction.[1] Patients with SCA are at higher risk of invasive bacterial infections,[2] especially those due to encapsulated germs,[3] leading to specific recommendations for antibiotics prophylaxis and vaccinations.[4,5] The main causes of infections are attributed to splenic dysfunctions,[6] complement activation defects,[7] genetic factors, and micronutrient deficiencies.[8] Reports from the literature have also suggested a central role in immune impairment, especially during vaso-occlusive crises (VOC). In septic shock, a clinical context characterized by an initial systemic inflammatory response, the down-regulation of human leukocyte antigen-DR expression on circulating monocytes (mHLA-DR) has been demonstrated. In this setting, mHLA-DR expression is considered a pertinent marker to identify patients with an increased risk of nosocomial infections and, therefore, of deleterious outcome.[9]

The present study evaluates the mHLA-DR expression in SCA patients during and after VOC.

Eighteen homozygous (HbSS) SCA adults with VOC, seen between October 2017 and April 2018 at the Edouard Herriot University Hospital in Lyon (France), were included in this one-center prospective study. A painful episode, defined as VOC, lasted for more than four hours; the patient felt that the pain was typical of that of vaso-occlusion, no other etiology of pain could be identified by the physicians, and the patient required hospitalization to the Emergency Department to treat the pain with opioids. SCA patients with VOC were compared to a control group, including 18 SCA subjects in clinical steady-state seen in the same institution over the same period. The study was conducted following the guidelines set by the Declaration of Helsinki. All patients gave written informed consent prior to inclusion. The study was approved by the "CPP Sud-Est IV" Ethics Committees (L16-47).

Venous peripheral blood was drawn to assay mHLA-DR expression and lymphocyte subsets counts (T-, B-, and NK-cell) on day 0 (D0) at crisis onset and then on day 1 (D1) and between day 3 and day 7 (D3-7). In 6 of the 18 patients, mHLA-DR expression was monitored again four months (M4) after crisis recovery. In the control cohort, peripheral venous blood was harvested during medical consultation. Blood was sent on ice to the immunology laboratory within 3 hours, and then the monocyte HLA-DR expression was assessed by flow cytometry using a standardized technique. Results are expressed as the number of antibodies bound per cell (AB/C).

Mann-Whitney tests were used to compare non-parametric biological values between patients in crisis and patients in steady-state (control cohort). In contrast, the Wilcoxon test was used to compare patients in crisis at different time points. Statistical analyses were performed using SPSS (IBM Statistics) and GraphPad Prism software. All *P* values were two-sided and statistical significance was defined as $P < 0.05$.

The baseline characteristics of the group in crisis and the control group are detailed in **Table 1**. Half of the patients with VOCpresented with fever (>38°C). Patients in crisis demonstrated significantly higher CRP levels ($P < 0.0001$), higher leukocytosis with higher neutrophil counts, and higher monocyte counts ($P< 0.0001$, $P = 0.0001$, and $P = 0.0048$, respectively) than patients from the control cohort. During hospitalization, acute chest syndrome (ACS) occurred in 3 patients (16.7%), and pneumonia (defined on radiological criteria) was diagnosed in 2 patients (11.1%). mHLA-DR expression of the control group was within the range

Table 1. Patient characteristics at baseline.

Patient characteristics	SCA in crisis N=18	SCA steady-state N=18	*P* value
Age, years*	27.22 +/- 7	34.5 +/- 8	< 0.01
Male / Female	12/6	6/12	< 0.05
Treatment			
Hydroxyurea	7 (38.9%)	8 (44.4%)	NS
Blood exchange transfusion	2 (11.1%)	4 (22.2%)	NS
G6PD deficit	1	0	NS
SCA Antecedents			
VOC ≥ 3/ year	13 (72.22%)	2 (11.1%)	< 0.001
Acute chest syndrome	11 (61.1%)	7 (38.9%)	NS
Organ damage			
Cerebral vasculopathy	2	1	NS
Retinopathy	1	5	NS
Heart disease	4	5	NS
Osteonecrosis	6	7	NS
Liver disease	2	2	NS
Kidney disease	2	2	NS
Ulcers	2	2	NS
Priapism	1	1	NS
Iron overload	3	6	NS
Cholecystectomy	5	10	NS
Biology			
C reactive protein (mg/L)	86.8 +/- 82	6.4 +/- 6.3	< 0.001
Lactate dehydrogenase (UI/L)	617.7 +/- 234	464 +/- 118.4	< 0.05
Hematological values			
White blood cell count (10^9/L)	14.7 +/- 4.8	8.9 +/- 2.8	<0.001
Neutrophils (10^9/L)	10.2 +/- 4.3	5 +/- 2.3	< 0.001
Lymphocytes (10^9/L)	2.8 +/- 1.7	2.8 +/- 0.9	NS
Eosinophils (10^9/L)	0.2 +/- 0.2	0.2 +/- 0.2	NS
Basophils (10^9/L)	0.1 +/- 0.1	0.1 +/- 0.1	NS
Monocytes (10^9/L)	1.3 +/- 0.4	0.8 +/- 0.4	< 0.01
Platelet count (10^9/L)	337.2 +/- 123.2	384.7 +/- 127.8	NS
Hemoglobin (g/dL)	8.7 +/- 1.2	9.4 +/- 1.4	NS
T-cell, B-cell, NK-cells(cell/µL)			
T lymphocytes	1188 +/- 711	1394 +/- 500	NS
$CD4^+$ T cells	783 +/- 521	933 +/- 388	NS
$CD8^+$ T cells	338 +/- 156	384 +/- 142	NS
NK cells	256 +/- 192	340 +/- 297	NS
B lymphocytes	642 +/- 682	658 +/- 376	NS

*Data expressed as mean +/- SD. NS= no significant difference.

of normal values. At the onset of VOC (D0), SCA patients had lower mHLA-DR expression than the control group ($P = 0.0001$). A lower level of mHLA-DR expression was maintained at D1 ($P = 0.052$), but the level increased by D3 ($P < 0.05$). mHLA-DR expressions returned to normal values compared to D0 ($P < 0.05$) when measured following VOC recovery (**Figure 1**). No significant differences were observed among the study and control groups regarding the absolute counts of total lymphocytes and lymphocyte subpopulations.

This study is the first evaluating mHLA-DR expression in SCA patients with VOC. The results showed a significant decrease in mHLA-DR levels in patients in crisis as compared to SCA subjects in clinical steady-state. The nadir was reached at VOC onset. The

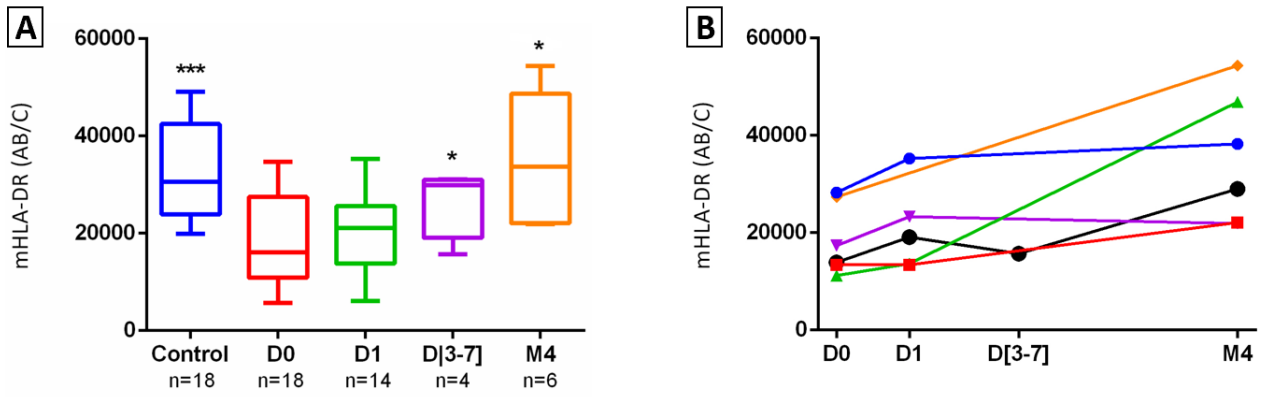

Figure 1. Monocyte HLA-DR expression overtime in SCA patients.
Monocyte HLA-DR expression was measured by flow cytometry at day 0 (D0, crisis onset), day 1 (D1, 24h after crisis), between days 3 and 7 (D3-7), and at 4 months after the crisis (M4). Eighteen SCA patients were included at VOC onset and 18 SCA patients in steady-state were used as controls. Results were expressed as numbers of antibodies bound per cell (AB/C).
(A). Results are presented as box-plots. Samples were: Control (N=18), D0 (N=18), D1 (N=14), D3-7 (N=4), and M4 (N=6). Comparisons between D0 and the others groups were achieved by using non-parametric Mann Whitney test. *P* values: *p<0.05, **p<0.01, ***p<0.001.
(B). Overtime evolution of mHLA-DR expression in the 6 SCA patients from the VOC group monitored until M4.

mHLA-DR levels remained low for 24 hours, then increased by D3, to finally normalized by a few months. Values observed in the VOC group were similar to data previously described in trauma patients.[10] However, they remained higher than those previously observed in patients with septic shock.[11]

In SCA patients, VOC is characterized by a systemic inflammatory response, which is associated with increased levels of biological markers (CRP and leukocytosis) and cytokines (such as IL-6, IL-8, IL-17, and TNF-α).[12,13,14] Prior findings showed that circulating monocytes display an activated phenotype for VOC. The mechanism involves the activation of the endothelium.[15] In the current study, circulating monocytes from SCA patients in crisis demonstrated an altered/anergic phenotype characterized by decreased MHC class II expression. Decreased HLA-DR expression has previously been proposed as a marker of immune alterations, and experimental ex-vivo studies have shown a linear correlation with altered TNF-α production by monocytes.[16] Similarly, it has been suggested in septic shocks that the decreased mHLA-DR expression reflects the homeostatic regulation of the immune response from initial overwhelming inflammation to secondary immunosuppression.[17]

Despite limitations due to the small number of patients, our first results suggest that, following the acute injury associated with VOC, a negative immunosuppressive feedback response occurs in SCA patients to compensate for the acute inflammatory response initiated during the VOC.[13] Furthermore, our findings are supported by previous data showing that plasma concentrations of immunoregulatory cytokines, such as IL-10, TGF-β, and PGE_2, are also increased in SCA patients during VOC.[14,18,19] Decreased HLA-DR expression is in accordance with Munford and Pugin's hypothesis[20] that every systemic inflammatory response is associated with a possible delayed immunosuppressive mechanism. Similarly, patients with severe sepsis secondarily develop a 'compensatory anti-inflammatory response syndrome' in reaction to inflammatory septic stress. Furthermore, the persistence of low mHLA-DR expression over time in patients with septic shock is associated with an increased risk of death or secondary infections.[11,21]

In the present study, the lowest levels of mHLA-DR expression at D0 were observed in the two patients who developed pneumonia (5738 AB/C and 9793 AB/C, respectively). In VOC setting, a low mHLA-DR expression could identify an underlying ongoing infection and contribute to an early identification of high-risk patients.

Overall, mHLA-DR expression significantly decreases in SCA patients during VOC occurrence. Further studies should be conducted to confirm these preliminary results and to establish relationships between putative immunosuppression and an increased risk for infection. Upon confirmation in larger cohorts, mHLA-DR may become an informative biomarker to monitor patients during the crisis, especially as its cost and feasibility are not limiting factors in developed countries. Unfortunately, this biomarker should not be easily affordable in most tropical African settings where most SCA patients reside.

Author Contributions. RF designed the study, analyzed and interpreted data, and drafted the manuscript; GM and FV provided immunologic data; GC provided patients and control cohort, participated in therapy decision-making and patient care, reviewed the manuscript, and gave final approval; PC and EN performed statistical analyses; AH designed the study, analyzed and interpreted data, participated to therapy decision making and patient care, reviewed the manuscript, and gave final approval. All authors approved the final manuscript.

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