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EDITORIAL

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Is the measurement of monocytes HLA-DR expression useful in patients with sepsis?

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Leukocytes undergo significant reprogramming in patients with sepsis [1]. Compared with monocytes from healthy subjects, monocytes from patients with severe infections lose their capacity to mount a pro-inflammatory response after stimulation with bacterial products. Not only do they produce less pro-inflammatory cytokines, they also increase their production of anti-inflammatory mediators and receptor antagonists, such as the monocyte deactivating interleukin (IL)-10 and IL-ra [2]. This gives the plasma a dominant "anti-inflammatory" flavor [3]. It has been proposed that this phenomenon is a compensatory reaction to the initial inflammatory response [4]. We believe that this is an essential adaptive systemic response aimed at concentrating the inflammatory response at the site of infection, in the organs [5]. A decreased surface expression of major histocompatibility class II molecules was also described in circulating monocytes from patients with sepsis, as well as in other critically ill patients. This is associated with an impaired antigen presentation capacity. The association of a decreased HLA-DR expression and a blunted pro-inflammatory cytokine production by monocytes is also known as "immunoparalysis" [6]. The molecular mechanisms of HLA-DR downregulation at the surface of monocytes from critically ill patients are now better understood. HLA-DR production is impaired, and is retained intracellularly [7, 8, 9, 10]. Interestingly, this monocyte phenotype can be reversed by the treatment of septic patients with interferon-γ or granulocyte–monocyte colony-stimulating factor [11, 12, 13]. Unfortunately, these studies were not powered to demonstrate an improvement in survival in patients with severe sepsis, nor did they show a convincing decrease in the rate of secondary infections.

Monocyte HLA-DR expression has been reported in 40 studies in a total of over 1,400 critically ill patients [14, 15]. An association between low HLA-DR expression and bad outcome was found in many of these studies, but not in all. Importantly, the populations of critically ill patients studied were quite diverse: medical, postsurgical, burns, transplant, and trauma patients, as well as patients with sepsis of various degrees of severity. The outcome variables measured were also numerous, including rate of secondary infections, development of shock, organ dysfunction and death. It has therefore been impossible to aggregate data from the different studies and to draw a definite conclusion as to whether HLA-DR measurement offers a significant benefit to the critically ill patient.

In a study reported in this issue of *Intensive Care Medicine*, Monneret et al. measured the level of expression of monocyte HLA-DR and its evolution with time in 93 patients with septic shock [16]. Persisting low HLA-DR values—defined as $< 30\%$ positive monocytes—at day 3–4 after ICU admission identified a population of patients with a remarkably high mortality rate. Low HLA-DR was an independent factor by multivariate analysis and was found to be a better predictor of mortality than SAPS II and SOFA scores. In the absence of a specific intervention on HLA-DR expression, it is impossible to say whether

low HLA-DR is a marker or a "mediator" of poor outcome in patients with septic shock. Monocyte HLA-DR levels may at best shed some light on the pathogenesis of immune dysfunction of critically ill patients. In no case will this marker—as many other markers or scores of severity—help the clinician to make radical decisions, such as whether to withhold or withdraw treatments, for example. Monocyte HLA-DR expression is, however, becoming an easy and robust marker of the immune dysfunction of critically ill patients. It could be used in the future both for the detection of immune paralysis and as a useful monitoring tool for interventions in immune-suppressed critically ill patients.

This study reminds us of the three essential steps necessary to define a biological marker as a useful marker in our daily ICU practice. Firstly, the analytical conditions of the test must be optimized, standardized and made reproducible. This has been a problem for many years with the measurement of HLA-DR expression. Inconsistent results rendered comparisons between centers virtually impossible. A kit for the quantification of the mean expression of HLA-DR in circulating monocytes is now commercially available, which has very significantly decreased intra- and inter-assay variability [17]. It requires access to a flow cytometer, however, and is therefore not exactly a simple bedside test. Monneret et al. measured HLA-DR with a non-commercial but accurate and reproducible method [18]. Interestingly, HLA-DR was significantly associated with outcome when these authors expressed results as % positive monocytes, but not when cell fluorescence was measured as "mean fluorescence index (MFI)". Although there is no clear explanation for this finding, this might only be due to an insufficient number of patients to achieve significant results with MFI. It would probably now be important to compare the method used by Monneret et al. with that of a commercially available quantification kit for HLA-DR expression with regard to their respective values as a prognostic marker.

Secondly, the parameter should be tested in normal subjects and prospectively in patients with well-characterized pathological conditions over time and with well-defined outcomes. This step is essential to determine normal values and also significant cut-off values at given time points during the course of the disease. The studies should be powered such that one can rely with sufficient confidence on the findings. HLA-DR expression in critically ill patients is a prototypical situation where the studies are lacking that might establish indisputable confidence in this marker in the clinical arena. The study by Monneret et al. does, however, add a piece to this edifice and is quite satisfying in this regard. The authors included a fairly homogeneous population and a reasonable number of septic shock patients surviving after 48 h, measured HLA-DR at several time points during the course of the disease, and determined a robust outcome, mortality. A methodological weakness of this study is its monocentric design, preventing testing of the inter-center reproducibility of the results. Furthermore, their findings now need to be independently validated in another cohort of similar patients.

The final and important step is the application of the test in "impact studies". No clinician wants another prognostic marker to test at the bedside without knowing what he has to do with the results. Measuring this parameter should potentially allow the clinician to modify the care and ideally the outcome of his patient.

As said earlier, it is not conceivable that low HLA-DR on day 3 or 4 will be used by the clinician to take drastic decisions, despite its good prognostic value. However, the results from this study permit identification of a population of patients with septic shock that have an increased risk of mortality, with an accuracy that is better than severity scores. It is very likely that monocyte HLA-DR expression persisting at low values at day 3 or 4 after the onset of septic shock will identify patients with a persistent immune dysfunction. These patients are probably those who fail to clear out their primary bacterial infection and those at risk of acquiring lethal secondary infections. Therefore, the identification of these patients might be valuable to reinitiate infectious work-ups, optimize the source control, and possibly modify the antibiotic therapy. This test may also prove very useful to select patients in future intervention trials who could profit from immunomodulatory treatments. Although promising as a future tool for the care of the critically ill patient, monocyte HLA-DR expression remains an investigational parameter, still has to be fully validated, and needs to find its niche before it is widely used.

References

1. Cavaillon JM, Adrie C, Fitting C Adib-Conquy M (2005) Reprogramming of circulatory cells in sepsis and SIRS. J Endotoxin Res 11:311–320
2. Cohen J (2002) The immunopathogenesis of sepsis. Nature 420:885–891
3. Cavaillon JM (2002) "Septic plasma": an immunosuppressive milieu. Am J Respir Crit Care Med 166:1417–1418
4. Bone RC (1996) Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 24:1125–1128
5. Munford RS, Pugin J (2001) Normal responses to injury prevent systemic inflammation and can be immunosuppressive. Am J Respir Crit Care Med 163: 316–321

6. Volk HD, Reinke P, Docke WD (2000) Clinical aspects: from systemic inflammation to 'immunoparalysis'. Chem Immunol 74:162–177
7. Pangault C, Le Tulzo Y, Tattevin P, Guilloux V, Bescher N, Drenou B (2006) Down-modulation of granulocyte macrophage-colony stimulating factor receptor on monocytes during human septic shock. Crit Care Med 34:1193–1201
8. Le Tulzo Y, Pangault C, Amiot L, Guilloux V, Tribut O, Arvieux C, Camus C, Fauchet R, Thomas R, Drenou B (2004) Monocyte human leukocyte antigen-DR transcriptional downregulation by cortisol during septic shock. Am J Respir Crit Care Med 169:1144–1151
9. Pachot A, Monneret G, Brion A, Venet F, Bohe J, Bienvenu J, Mougin B, Lepape A (2005) Messenger RNA expression of major histocompatibility complex class II genes in whole blood from septic shock patients. Crit Care Med 33:31–38 discussion 236–237
10. Fumeaux T, Pugin J (2002) Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. Am J Respir Crit Care Med 166:1475–1482
11. Nierhaus A, Montag B, Timmler N, Frings DP, Gutensohn K, Jung R, Schneider CG, Pothmann W, Brassel AK, Schulte Am Esch J (2003) Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis. Intensive Care Med 29:646–651
12. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W (1997) Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med 3:678–681
13. Nakos G, Malamou-Mitsi VD, Lachana A, Karassavoglou A, Kitsiouli E, Agnandi N, Lekka MF (2002) Immunoparalysis in patients with severe trauma and the effect of inhaled interferon-gamma. Crit Care Med 30:1488–1494
14. Spittler A, Roth E (2003) Is monocyte HLA-DR expression predictive for clinical outcome in sepsis? Intensive Care Med 29:1211–1212
15. Tschoeke SK, Moldawer LL (2005) Human leukocyte antigen expression in sepsis: what have we learned? Crit Care Med 33:236–237
16. Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, Thizy H, Bienvenu J, Gueyffier F, Vanhems P (2006) Intensive Care Med DOI 10.1007/s00134–0204-8
17. Docke WD, Hoflich C, Davis KA, Rottgers K, Meisel C, Kiefer P, Weber SU, Hedwig-Geissing M, Kreuzfelder E, Tschentscher P, Nebe T, Engel A, Monneret G, Spittler A, Schmolke K, Reinke P, Kunz D (2005) Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. Clin Chem 51:2341–2347
18. Monneret G, Elmenkouri N, Bohe J, Debard AL, Gutowski MC, Bienvenu J, Lepape A (2002) Analytical requirements for measuring monocytic human lymphocyte antigen DR by flow cytometry: application to the monitoring of patients with septic shock. Clin Chem 48:1589–1592