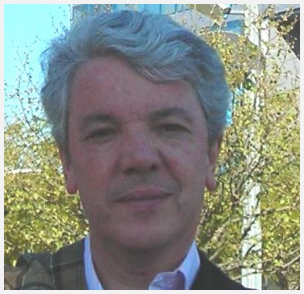
ImmunoTools *special* Award 2014



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The cytokine response profile of immunosuppressed sepsis patients

Backgound

Sepsis patients typically exhibit a biphasic immune response to infections. Survivors of an initial hyper-immune state, characterized by widespread inflammatory tissue injury, subsequently manifest a state of immune hypo-responsiveness that is characterized by the desensitisation of leukocytes to Toll-like receptor (TLR) ligands, resulting in their inability to induce an appropriate pro-inflammatory response on subsequent exposure to pathogens and thus to clear infections. This predisposes these patients to 'second-hit' infections, which have a high mortality rate and, even when not fatal, result in numerous adverse outcomes including prolonged periods of costly organ support. This state of immunosuppression is ill-defined and poorly understood, despite representing a more promising avenue of investigation for an immunomodulatory therapy than the initial hyper-immune phase, which is usually well-established before a therapeutic window presents itself.

The focus of the proposal on late-phase sepsis-induced immunosuppression represents a departure from the bulk of sepsis research, which has aimed at inhibiting rather than augmenting the inflammatory response. This strategy has largely been unsuccessful and there is a clear need for an effective immunomodulatory therapy for sepsis. This need coincides with the recent observation that, due to improvements in patient management, the majority of sepsis mortality is now due not to immune hyperactivation during the acute phase of the illness but rather due to immunosuppression and failure to clear secondary infections during the later phase[1,2]. The proposal also builds on findings from our laboratory recently published in *Science, Translational Medicine* showing that modulating the TLR co-receptor activity of

CD14 can amplify immune responses to pathogens, including those of immunosuppressed sepsis patients[3].

Aim and objective

The award will help to conduct preliminary work to better define the immunosuppression state of sepsis patients. Specifically, we plan to test whether the pro-inflammatory cytokine response profile of immunosuppressed sepsis patients can define patient subgroups at higher risk of developing secondary infections and thus serves as a prognostic biomarker signature.

Plan of Investigation

A cohort of 100 patients with severe sepsis and septic shock admitted to the participating intensive care units (ICUs) will be recruited, together with a matched cohort of 50 healthy controls. A complete clinical dataset will be recorded, with particular reference to the incidence of secondary infections (positive microbiological cultures and clinically defined infection). Access to these populations and the relevant ethical permissions are in place.

Whole blood samples will be taken from patients on admission to the ICUs. The plasma levels at baseline of a panel of 7 pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, IFNγ and TNFα) will be tested in the samples (ELISA). The whole blood samples will be challenged with microbial components and bacteria that activate the two major TLRs that sense bacteria, TLR2 and TLR4: Pam_3-Cys-Ser-Lys_4 (synthetic triacyl lipopeptide and TLR2/1 ligand), Lipopolysaccharide (LPS, a TLR4 ligand); and heat-killed Gram-positive (*L. monocytogenes*) and Gram-negative (*E. coli*) bacteria. The cytokine response to this microbial challenge will be evaluated by ELISA.

In order to characterise the cytokine response profile of sepsis-induced immunosuppression, the patients' cytokine profile at baseline and after microbial challenge will be compared with that of healthy controls. Data will be analysed on the basis of: 1) the amplitude of individual cytokine responses, 2) differential cytokine responses and 3) reactivity to different microbial challenges. Patient subgroup analysis will be performed on the basis of cytokine profiles, and multivariate regression analysis will be used to test for associations between cytokine response and the development of secondary infections (predicted n=25, patients that develop secondary infections).

Expected outcomes

It is anticipated that this preliminary study, which will be followed by a larger study cohort, will help to improve on current management and therapies for immunosuppressed sepsis patients.

Key references

1. Otto GP, Sossdorf M, Claus RA, Rodel J, Menge K, Reinhart K, Bauer M, Riedemann N. 2011.The late phase of sepsis is characterised by an increased microbiological burden and death rate. *Crit Care*, 15: R1832.
2. Meisel C, Schefold JC, Pschowski R, Baumann R, Hetzger K, Gregor K, Weber-Carstens S. 2009. Granulocyte–Macrophage Colony-stimulating Factor to Reverse Sepsis-associated Immunosuppression A Double-Blind, Randomized, Placebo-controlled Multicenter Trial. *Am J Respir Crit Care Med*, 180: 640-648.
3. Targeting the TLR co-receptor CD14 with TLR2-derived peptides modulates immune responses to pathogens. A.-C. Raby, B. Holst, E. Le Bouder, C. Diaz, E. Ferran, L. Conraux, J.-C. Guillemot, B. Coles, A. Kift-Morgan, C. S. Colmont, T. Szakmany, P. Ferrara, J. E. Hall, N. Topley, M. O. Labéta, *Sci. Transl. Med*. 5, 185ra64 (2013).