British Journal of Anaesthesia **111** (5): 778–87 (2013)
Advance Access publication 10 June 2013 · doi:10.1093/bja/aet205

BJA

CRITICAL CARE

Combined dysfunctions of immune cells predict nosocomial infection in critically ill patients

A. Conway Morris[1,6]*, N. Anderson[2], M. Brittan[1], T. S. Wilkinson[1,3], D. F. McAuley[4], J. Antonelli[5], C. McCulloch[5], L. C. Barr[1], K. Dhaliwal[1], R. O. Jones[1], C. Haslett[1], A. W. Hay[6], D. G. Swann[6], I. F. Laurenson[7], D. J. Davidson[1], A. G. Rossi[1], T. S. Walsh[1,6] and A. J. Simpson[1,8]

[1] MRC/University Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, UK
[2] Centre for Population Health Sciences, The University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK
[3] Institute of Life Science, Microbiology and Infection, College of Medicine, Swansea University, Swansea SA2 8PP, UK
[4] Centre for Infection and Immunity, Queen's University of Belfast, Belfast BT9 7BL, UK
[5] Clinical Research Facility, [6] Critical Care, [7] Clinical Microbiology, NHS Lothian, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, UK
[8] Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

* Corresponding author. E-mail: mozza@doctors.org.uk

Editor's key points

- This study found a temporal occurrence of immune dysfunction across T-cells, monocytes, and neutrophils, and proved their relationship with the development of nosocomial infection in ICU patients.
- This immune dysfunction is not an 'all or nothing' response and can affect different cell types at different times.
- This study does not support the recent suggestion that immune dysfunction is restricted to patients with sepsis.

Background. Nosocomial infection occurs commonly in intensive care units (ICUs). Although critical illness is associated with immune activation, the prevalence of nosocomial infections suggests concomitant immune suppression. This study examined the temporal occurrence of immune dysfunction across three immune cell types, and their relationship with the development of nosocomial infection.

Methods. A prospective observational cohort study was undertaken in a teaching hospital general ICU. Critically ill patients were recruited and underwent serial examination of immune status, namely percentage regulatory T-cells (Tregs), monocyte deactivation (by expression) and neutrophil dysfunction (by CD88 expression). The occurrence of nosocomial infection was determined using pre-defined, objective criteria.

Results. Ninety-six patients were recruited, of whom 95 had data available for analysis. Relative to healthy controls, percentage Tregs were elevated 6–10 days after admission, while monocyte HLA-DR and neutrophil CD88 showed broader depression across time points measured. Thirty-three patients (35%) developed nosocomial infection, and patients developing nosocomial infection showed significantly greater immune dysfunction by the measures used. Tregs and neutrophil dysfunction remained significantly predictive of infection in a Cox hazards model correcting for time effects and clinical confounders {hazard ratio (HR) 2.4 [95% confidence interval (CI) 1.1–5.4] and 6.9 (95% CI 1.6–30), respectively, $P=0.001$}. Cumulative immune dysfunction resulted in a progressive risk of infection, rising from no cases in patients with no dysfunction to 75% of patients with dysfunction of all three cell types ($P=0.0004$).

Conclusions. Dysfunctions of T-cells, monocytes, and neutrophils predict acquisition of nosocomial infection, and combine additively to stratify risk of nosocomial infection in the critically ill.

Keywords: cross infection; intensive care; lymphocytes; neutrophils

Accepted for publication: 22 April 2013

Many diseases that can precipitate the need for exogenous organ support and admission to intensive care are characterized by a profound systemic inflammatory response,[1] with associated immune cell activation,[2] and immune system-mediated organ damage.[3] However, it is now increasingly apparent that this over-exuberant inflammation is accompanied by an equally vigorous counter-regulatory anti-inflammatory response.[4]

The anti-inflammatory response to the systemic inflammatory response syndrome manifests across a range of cellular actions and functions, involving both the innate and adaptive arms of the immune system.[4] Defects have been noted in neutrophils,[5–8] monocytes,[9] T lymphocytes,[10] and B lymphocytes and also splenic dendritic cells.[11 12]

The recent identification of elevated levels of regulatory helper-T cells (Tregs) in sepsis[13] is in keeping with the supposition that much of the immunosuppression arises from the overactivation of counter-regulatory mechanisms. In human and experimental sepsis, Tregs impair the proliferative response of lymphocytes.[14]

The demands of organ support require the disruption of physical and physiological barriers through the placement of devices such as endo-tracheal tubes. It is thought that the combination of immune vulnerability and such routes of microbial colonization are responsible for the high rates of nosocomial infection seen in critically ill patients.[15] These secondary infections typically occur in 25–35% of those admitted to intensive care units (ICUs),[15] a rate that approaches that seen in neutropaenia.[16] These infections are associated with increased length of stay,[17] morbidity[18] and mortality,[19] and therefore are of considerable concern to patients and clinicians. Although it seems plausible that the immune defects found in critical illness are associated with the acquisition of nosocomial infection, there is little published evidence for this, and what data there are concentrate on single types of immune cell. Furthermore, the temporal relationship between immune dysfunction and nosocomial infection is not always clear,[6] limiting any inferences regarding causality.

This study aimed to characterize the temporal patterns of three measures of immune dysfunction, sampling both the innate and adaptive arms of the immune system, and to derive potential new biomarkers of susceptibility to nosocomial infection. The cell types and measures of dysfunction chosen were the level of Tregs as a percentage of all CD4+ lymphocytes,[14] monocyte deactivation assayed by monocyte HLA-DR expression[9] and C5a-mediated neutrophil dysfunction assayed by surface CD88 expression.[6 8]

Methods

Reagents

Fluorescein isothiocyanate (FITC)-conjugated murine anti-human CD4, allophycocyanin-conjugated murine anti-human CD25, and phycoerythrin (PE)-conjugated murine anti-human FOXP3 antibodies were obtained from eBioscience (San Diego, CA, USA). Red cell lysis buffer, fixation/permeabilization solution, and flow staining buffer were obtained from eBioscience. Alexa Fluor™ 647-conjugated murine anti-human CD88 antibodies were obtained from AbD Serotec (Abingdon, UK), and QuantiBRITE monocyte HLA-DR assay was obtained from Becton Dickson Biosciences (Oxford, UK). Tri-colour-conjugated murine anti-human CD16 and CD62L, FITC-conjugated murine anti-human CD11b and CD14, and PE-conjugated murine anti-human CD3 and CD64 were obtained from Invitrogen (Paisley, UK).

Volunteers, patients, and setting

Healthy volunteers were recruited from University of Edinburgh staff, to act as a reference group for the cellular markers examined.

The clinical study took place in an 18-bed teaching hospital medical-surgical ICU.

Critically ill patients, defined as those admitted to ICU and requiring support of one or more organ systems (invasive ventilation; requirement for vasopressors, inotropes, or both haemofiltration) and predicted to require such support for 48 h or more, were screened for recruitment. Exclusion criteria were: age <16; pregnancy; known human immunodeficiency virus infection; known in-born errors of neutrophil metabolism; haematological malignancy; use of immunosuppressive drugs other than corticosteroids; and those thought unlikely to survive for >24 h. Patients were also excluded if they were involved in another study that involved blood sampling, or if they had suspected novel (A/H1N1) influenza. Informed consent was obtained directly from patients where possible, otherwise informed consent was obtained from the next of kin. Clinical data were collected regarding potential risk factors for nosocomial infection,[15] these data included 'shock', defined by requirement for norepinephrine, epinephrine, dobutamine infusion, or both. Ethylenediaminetetraacetic acid anti-coagulated blood was collected at study enrolment (within 48 h of ICU admission), then at study Day 2, Days 3–4, and Days 6–10 unless a study endpoint was achieved. Study endpoints were ICU-acquired infection (see Supplementary material for definition); death without ICU-acquired infection; or discharge from ICU without ICU-acquired infection.

Details of flow cytometric protocols and analysis for determination of immune dysfunction are included in the Supplementary material.

Infections

Diagnostic criteria were pre-defined for the major ICU-acquired infections, namely ventilator-associated pneumonia (VAP), blood stream infection (BSI), vascular catheter-related infection (CRI), urinary tract infection (UTI) and surgical site/soft tissue infections, based on those from the HELICS programme[20] (see Supplementary material for details). Data on infections were recorded by the study nurses (J.A. and C.M.), who were blinded to the immune phenotype. Day of infection was defined as the day on which positive microbial culture was obtained from the patient.

Where infection was strongly clinically suspected but did not fulfil HELICS criteria (for instance when cultures were taken while on antibiotics, cultures, or both were negative/equivocal), an expert panel (I.F.L., A.W.H., D.G.S., T.S.W., and A.J.S.), blinded to the immune phenotype, reviewed patients' data and the presence or absence of infection was adjudicated. In the absence of positive culture the day of infection was defined as the day of clinical deterioration. The adjudication outcome could be 'confirmed', 'probable', or 'unlikely' infection. Details of diagnostic criteria and expert panel adjudication procedures are set out in the Supplementary material.