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Transforming growth factor β-1 and interleukin-17 gene transcription in peripheral blood mononuclear cells and the human response to infection

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ABSTRACT

Introduction: The occurrence of severe sepsis may be associated with deficient pro-inflammatory cytokine production. Transforming growth factor β-1 (TGFβ-1) predominantly inhibits inflammation and may simultaneously promote IL-17 production. Interleukin-17 (IL-17) is a recently described pro-inflammatory cytokine, which may be important in auto-immunity and infection. We investigated the hypothesis that the onset of sepsis is related to differential TGFβ-1 and IL-17 gene expression. *Methods:* A prospective observational study in a mixed intensive care unit (ICU) and hospital wards in a university hospital. Patients (59) with severe sepsis; 15 patients with gram-negative bacteraemia but without critical illness and 10 healthy controls were assayed for TGFβ-1, IL-17a, IL-17f, IL-6 and IL-1β mRNA in peripheral blood mononuclear cells (PBMC) by quantitative real-time PCR and serum protein levels by ELISA. *Results:* TGFβ-1 mRNA levels are reduced in patients with bacteraemia and sepsis compared with controls ($p = 0.02$). IL-6 mRNA levels were reduced in bacteraemic patients compared with septic patients and controls ($p = 0.008$). IL-1β mRNA levels were similar in all groups, IL-17a and IL-17f mRNA levels are not detectable in peripheral blood mononuclear cells. IL-6 protein levels were greater in patients with sepsis than bacteraemic and control patients ($p < 0.0001$). Activated TGFβ-1 and IL-17 protein levels were similar in all groups. IL-1β protein was not detectable in the majority of patients. *Conclusions:* Down regulation of TGFβ-1 gene transcription was related to the occurrence of infection but not the onset of sepsis. Interleukin-17 production in PBMC may not be significant in the human host response to infection.

1. Introduction

In humans with severe sepsis a profound and complex immune paresis exists in the presence of systemic inflammation. The cytokine basis of this immune paresis is multifactorial, associated with attenuated TNFα gene expression, reduced interleukin-12 and interferon gamma gene expression, and excess interleukin-10 gene expression [1]. Intriguingly an increased risk for mortality in patients with infection has been related to excessive IL-10 production in relation to TNFα production [2]. Whilst it is acknowledged that this response pattern may be inherited [3], the precise genetic basis underlying it has yet to be identified.

A recent report from our group has identified IL-23 and IL-27 gene transcription in peripheral blood mononuclear cells as important regulators of the human host response to infection [4]. These cytokines are both members of the IL-12 family of cytokines and may be of greater importance than IL-12 in influencing an appropriate bactericidal response to infection. Furthermore deficient IL-23 and excessive IL-27 were associated with the occurrence and outcome of sepsis. Whereas both IL-23 and IL-27 have recently been described as antagonistic regulators of CD4+ T cells that have a Th17 phenotype, it remains unclear as to whether the link between IL-23 and IL-27 with outcomes in sepsis, is mediated by this IL-17 cytokine pathway or alternatively by regulation of interferon gamma and TNFα gene expression [5].

Abbreviations: TGFβ-1, transforming growth factor β-1; IL, interleukin; ICU, intensive care unit; mRNA, messenger ribonucleic acid; PBMC, peripheral blood mononuclear cells; qRT-PCR, quantitative real-time polymerase chain reaction; ELISA, enzyme linked immunosorbent assay; TNFα, tumor necrosis factor α; IFNγ, interferon-γ; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment; EDTA, ethylenediaminetetraacetic acid; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; cDNA, complementary deoxyribonucleic acid; ABI, applied biosystems; Ct, threshold cycle; pg/mL, picogram per millilitre; *n*, number; C5, complement 5.

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IL-17a and IL-17f are pro-inflammatory cytokines produced by macrophages and a specific subgroup of CD4 cells. Recently much emphasis has been placed on their importance in the pathogenesis of immune mediated encephalitis in animals, and these cytokines appear to have a protective effect in animal models of bacterial infection [6,7]. In naïve CD4 cells it was reported that differentiation into a Th17 lineage is driven by TGFβ-1. This was shown to be crucially dependent on IL-6 and IL-23, with IL-27 inhibiting the effects of IL-23. Animal models of encephalitis also suggest that Interleukin-1β may also be an important factor in promoting IL-17 production [8].

However, cytokines involved in regulating IL-17 production are pleiotrophic and although they may be important in the immune response to infection, it is unclear whether they exert their dominant effect by means of the IL-17 cytokine family. In this regard, TGFβ-1 is a potent regulatory cytokine with diverse hemopoietic functions. While the key function of TGFβ-1 in the immune system is to maintain tolerance through regulation of differentiation, proliferation and survival of lymphocytes, TGFβ-1 also moderates interferon gamma production by natural killer cells, and in addition TGFβ-1 promotes a CD4Th1 response to infection [9,10]. Thus TGFβ-1 potentially has both anti-inflammatory and pro-inflammatory properties.

This study was performed to determine whether the human response to infection was related to TGFβ-1 and IL-17 gene transcription. We also assessed gene transcription and protein levels of recognised IL-17 regulatory cytokines by quantitative real-time PCR and ELISA. The data presented in this study provides new information regarding the pathogenesis of the human inflammatory response in infection and sepsis.

2. Methods

2.1. Patients

This observational study was conducted in St. James's Hospital, Dublin, Ireland, and was approved by the institutional ethics committee. Informed written consent was obtained from each patient or a relative. A total of 59 consecutive patients presenting with severe sepsis or septic shock, as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [11], as a primary admission diagnosis were enrolled over 12 months. All ICU patients received similar standardised care.

Severity of illness was characterised on admission to ICU using the simplified acute physiology score (SAPS2) [12] and the sequential organ failure assessment (SOFA) [13] scoring systems and again on day 7 with the SOFA score.

Individual clinical and laboratory variables were collected on day 1 and day 7 of ICU stay. The recorded variables represented the most significant derangements from normal values recorded over each 24-h period. The source of infection necessitating the ICU admission, ICU death or survival to ICU discharge was recorded. Fifteen consecutive patients admitted to general hospital wards, with a documented gram-negative bacteraemia, confirmed on blood culture were identified by the microbiology department and were assessed for enrolment in this study. Patients with such an infection but no associated or pre-existing organ failure or impending septic crisis were enrolled. Ten healthy staff members served as a control group.

2.2. Exclusion criteria

Exclusion criteria included: (1) pre-existing overt organ failure; (2) infection with the human immunodeficiency virus; (3) patients neutropenic as a result of chemotherapy; (4) patients receiving long-term treatment with corticosteroids; (5) trauma and burns patients; (6) non-Irish Caucasian ethnic background.

2.3. Blood sampling

Blood sampling was carried out within the first 24 h of ICU admission and again 7 days later. In bacteraemic patients, blood sampling was carried out within 24 h of the positive blood culture being reported. Blood samples were collected from healthy controls at one time point. Peripheral blood mononuclear cells (PBMCs) were immediately purified by density gradient centrifugation of EDTA anticoagulated blood using lymphoprep (Nycomed Pharma, Oslo, Norway). Serum was obtained from whole blood clotted for 30 min.

2.4. Total RNA extraction and reverse transcription

Total RNA was isolated from lysed PBMC using a commercially available kit (Qiagen) following the manufacturers instructions. In order to avoid amplification of contaminating genomic DNA, all samples were treated with RNase-free DNase (Qiagen) for 15 min. The quantity and purity of extracted RNA was measured by spectrophotometry (Eppendorf BioPhotometer, Eppendorf AG, Hamburg, Germany).

Total RNA was then reverse transcribed to cDNA as follows: 11.15 μl of water containing 500 ng of total RNA was first incubated at 65 °C for 10 min. The reverse transcription mix (18.85 μl) containing the following components were added: (1) 3 μl 0.1 M DTT; (2) 4.5 μl dimethyl sulfoxide: (3) 2 μl 100 μM Random Primers (Invitrogen Corp, Carlsbad, CA, USA); (4) 1.25 μl Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen Corp); (5) 6 μl 5XFirst Strand Buffer (Invitrogen Corp); (6) 1.5 μl 4 mM deoxynucleotide triphosphate mix (Promega Corp, Madison, MI, USA); (7) 0.6 μl 10 U/μl RNasin (Promega Corp). The samples were then incubated at 37 °C for 1 h.

2.5. Primers and probes

All primer and probes used in this study were synthesized at Applied Biosystems (Foster City, CA, USA). β-Actin, IL-6, TGFβ-1, IL-17A, IL-17F and IL-1β primers and probes were designed and customised (sequences listed in Appendix 1) as per Stordeur et al. [14].

2.6. Real-time PCR

The PCR reactions were carried out in an ABI Prism 7000 (Applied Biosytems). All reactions were performed either in triplicate or in duplicate. Thermocycling was carried out in a 20 μl final volume containing: (1) water up to 20 μl; (2) 10 μl Mastermix (Applied Biosystems); (3) 1, 2 or 3 μl of 6 pmol/μl forward and reverse primers (final concentration 300, 600 or 900 nM, see Appendix 1); (5) 1 μl of 4 pmol/μl Taqman Probe (final concentration 200 nM) or 1 μl of pre-customised primer/probe mix with default primer and probe concentrations (Appendix 1); (6) 0.8 μl standard dilution or 2.4 μl cDNA. After an initial denaturation step at 95 °C for 10 min, temperature cycling was initiated. Each cycle consisted of 95 °C for 15 s and 60 °C for 60 s, the fluorescence being read at the end of this second step. In total, 40 cycles were performed.

2.7. Standard curves and expression of the results

The DNA standards for β-Actin, TGFβ-1, IL-17A, IL-17F, IL-1β and IL-6 consisted of a cloned PCR product that included the quantified amplicon prepared by PCR from a cDNA population containing the target mRNA. Detailed information on these standards is given in Appendix 2. In order to quantify transcript levels a standard curve was constructed, for each PCR run, for each selected mRNA target from serial dilutions of the relevant standard to 10^1 copy numbers.