Monocyte CD 40 expression in sepsis and the effect of interferon gamma administration on outcome

Thesis submitted for partial fulfilment of doctorate degree in Critical Care Medicine

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Abstract

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Introduction: Sepsis induces an early inflammatory cascade initiated by the innate immune response. This often results in the development of multisystem organ failure. CD40 is a cell surface protein belonging to the tumor necrosis factor (TNF) receptor family. Ligation of monocyte CD40 by the T cell-derived CD40 ligand can trigger the production of various mediators, the transcription and activation of enzymes, and the upregulation of costimulatory molecules involved in the pathogenesis of sepsis. Interferon gamma (INF-γ) is a major activator of monocytes that increase their antigen presenting capacity. Administration of (INF-γ) has been shown to decrease infection. The aim of this work was to test the CD40 expression on monocyte as an objective tool to detect sepsis in ICU patients and the effect of (INF-γ) administration on improving survival.

Methods: The study included 60 patients who were admitted to the ICU with the diagnosis of sepsis (Dec 2003 - March 2004 and Sept - Nov 2004) and 15 normal healthy volunteers.. Blood was collected every day to perform flow cytometry analysis of the circulating monocytes in a lysis, no wash direct staining technique with quantification of fluorescence using the median fluorescence index (MFI). The patients were blindly randomized into 2 groups: one receiving conventional treatment of sepsis and the second receiving (INF-γ) 100µg daily by subcutaneous injection together with the conventional treatment of sepsis.

Results: The study included 60 patients (23 medical, 37 surgical), of whom 21 were female (36%), with a mean age of 63±17 years. The overall 28-day mortality was 30 % (18/60). The mean of CD40 expression on surface of monocytes of septic patients was 7.3 ± 0.6. The ROC curve for CD40 showed a specificity of 70%, a sensitivity of 75%, with an area under the curve (AUC) of 0.82, positive predictive value of 57% and negative predictive value of 81% at the level of 6.4. Administration of (INF-γ) in the second group of patients did not improve the survival (8/30, 26%) compared to placebo group (10/30, 33%), p= 0.3.

Conclusion: CD40 can be used to detect septic patients among the critically ill patients. Interferon-gamma administration was not successful in decreasing mortality among septic patients.

Key words: CD40, monocytes, sepsis, interferon gamma

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TNF-α	:	Tumor necrosis factor-alpha
IL	:	Interleukin.
TGF-β	:	Tumor growth factor-beta.
SIRS	:	Systemic inflammatory response syndrome
CARS	:	Compensatory anti-inflammatory response syndrome
HLA	:	Human leukocyte antigen.
APCs	:	Antigen presenting cells.
CD	:	Cluster of differentiation.
PMNs	:	Polymorph-nuclear leukocytes.
MHC	:	Major-histocompatibility-complex
NF-κB	:	Nuclear factor- kappa beta
TLRs	:	Toll like receptors.
MASP1	:	Mannan-binding lectin–associated proteases 1
IRAK	:	Interleukin-1 receptor–associated kinase
TRAF-6	:	Tumor necrosis factor–associated factor 6
MAP3K	:	Mitogen-activated protein kinase kinase kinase.
PAMPs	:	Pathogen-associated molecular patterns
PRR	:	Pattern-recognition receptors.
FcγR	:	Fc gamma receptor.
Fab	:	Antigen-binding fragments.
LFA-1	:	Lymphocyte-function–associated antigen 1
Ig	:	Immunoglobulin
Th1	:	T helper cell type 1
MOF	:	Multi-organ failure
MODS	:	Multi-organ dysfunction syndrome.
PAF	:	Platelet-activating factor.
ROS	:	Reactive oxygen species
NO	:	Nitric oxide
PLA2	:	Phospholipase A2

AA	:	Arachidonic acid
PG	:	Prostaglandin
TXA2	:	Thromboxane A2
RBCs	:	Red blood corpuscles
DIC	:	Disseminated intravascular coagulation
TFPI	:	Tissue factor pathway inhibitor
LPS	:	Lipopolysaccharide
GH	:	Growth hormone.
CIP	:	Critical illness polyneuropathy
PMT	:	Photomultiplier tube
ADC’s	:	Analog to digital converters
PE	:	Phyco-erythrin
FLS	:	Forward Light Scatter
ICU	:	Intensive care unit
HIV	:	Human immunodeficiency virus
NICUS	:	Nosocomial ICU sepsis
CDC	:	Center of disease control
SCCM	:	Society of critical care medicine
ESICM	:	European society of intensive care medicine
ATS	:	American thoracic society
APACHE II	:	Acute Physiology And Chronic Health Evaluation II
SOFA	:	Sequential organ assessment score.
FITC	:	Fluorescein iso-thio-cyanate
PerCP	:	Peridinin chlorophyll
TCR	:	T cell receptor
WBCs	:	White blood cells
MPO	:	Methyl peroxidase
GM-CSF	:	Granulocyte- monocyte colony stimulating factor.
NIF	:	Neutrophil inhibitory factor
MIF	:	Monocyte inhibitory factor

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Aim of The Work

The aim of this work is to study selected immune markers (which represent both innate and adaptive immune systems) on the surface of white blood cells of critically ill patients who are expected to stay for a relatively long period in the ICU

With the primary endpoints are trials to identify:

1. A marker that can predict the development of nosocomial sepsis in the ICU (NICUS) patients while their stay in the ICU.

2. A marker that can separate septic from non septic patients immediately after admission to the ICU.

The secondary end point is: Trying to identify a marker that can predict the development of mortality among the ICU patients.

Introduction

Despite important advances in critical care medicine during the last decades, the mortality rate of sepsis has remained high. Recent studies have shown that sepsis is a bimodal entity. The first phase is characterized by the systemic release of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-8, and by activation of the complement and coagulation cascades. In the second phase, anti-inflammatory mediators such as TGF-β, IL-10, Prostaglandin E_2 may be released in an effort to counteract ongoing inflammation. Depending whether pro or anti-inflammatory responses predominates, some people referred to a systemic inflammatory response syndrome (SIRS) and a compensatory anti-inflammatory response syndrome (CARS). [(1)]

The development of an adequate immune response to bacterial challenge relies on the complex interplay between the innate and specific (adaptative) immune systems. The innate immune system is dedicated to recognition of pathogens using invariant markers. Monocyte & macrophage functions include the recognition, uptake & killing of invading organisms in order to initiate an immune response then to present antigen to specific T lymphocytes to initiate the adaptative immune response. This initiation may be accomplished primarily by the Major Histo-compatibility Complex of class II comprising HLA-DR, HLA-DP, HLA-DQ, which are constitutively expressed on antigen presenting cells (APCs), with the help of costimulatory molecules such as CD40, CD80/86 and the secretion of pro-inflammatory mediators, such as TNF, IL-1, IL-12. [(2, 3)]

Studies have shown a decreased expression of HLA-DR on monocytes in patients with sepsis constitutes a marker of immune-paralysis. These patients might benefit from immune-stimulants, while patients with severe sepsis and normal or high monocyte HLA-DR expression might benefit from anti-inflammatory strategies. [1] Several groups have reported an increase in serum Soluble CD14 concentrations in human gram-negative and gram-positive septic shock with an associated increased mortality. [4]

On the other hand, neutrophils (PMNs) functions have been shown to follow a bimodal response during severe sepsis or septic shock with an increased expression of the functional molecule, eventually followed by a depression. During sepsis, stimulation of PMNs enhances interaction with endothelial cells [5] and PMNs appear to posses an activated phenotype as judged by an increased expression of adhesion molecule CD11b [6] and decreased expression of L-selectin. [7] Recently, it was noted that most PMNs that bind to endothelial monolayer express CD64.

Regarding the lymphocytes, many groups [8, 9] have described increase in the expression of CD 69 and DR during sepsis, which are considered activation markers of the T lymphocytes.

Chapter I

The Immune System

The immune system is an organization of cells and molecules with specialized roles in defending against infection. There are two fundamentally different types of responses to invading microbes. Innate (natural) responses occur to the same extent however many times the infectious agent is encountered, whereas acquired (adaptive) responses improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular components of innate responses include complement, acute-phase proteins, and cytokines such as the interferons. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, called antigen-presenting cells, display the antigen to lymphocytes and collaborate with them in the response to the antigen. B cells secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells help B cells to make antibody and can also eradicate intracellular pathogens by activating macrophages and by killing virally infected cells. Innate and acquired responses usually work together to eliminate pathogens. [(10)]

All these cells develop from pluripotent stem cells in the fetal liver and in bone marrow and then circulate throughout the extracellular fluid. B