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RESEARCH ARTICLE

Monocyte Migration and Inflammatory Response in the Pathogenesis of Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects multiple synovial joints. Monocytes/Macrophages accumulate in synovium, where they play a central role in inflammation and joint destruction. In human peripheral blood, two monocyte subpopulations with distinct functional properties have been defined by their expression of CD14 and CD16 molecules. In the present study, we investigated the relevance of CD14+, CD16+, MIF and TLR4 expression in blood monocytes in RA population. Flowcytometric analysis was done for different monocytes by staining with CD14, CD16, MIF and TLR4 tagged with flurochromes in order to assay the inflammatory cytokines from 257 RA patients and 270 healthy controls. Inflammatory monocytes (CD14+ and CD16+) population was significantly high in subjects with RA than the healthy control. mCD14, mTLR4, CD16 and MIF expression levels were also increased the susceptibility of RA. The study also found that expression level of TLR4, CD14, MIF and CD16 can be used as a diagnostic biomarker for RA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects multiple synovial joints. Synovial infiltration with cells of the monocyte/macrophage lineage is thought to be responsible for both the chronic inflammation and the joint destruction (Burmester, et al. 1997). Of importance, activated macrophages are the major source of many cytokines in the joint, including tumor necrosis factor α (TNF α), IL-1, IL-8, and granulocyte–macrophage colony-stimulating factor (Feldmann, et al. 1996). These cytokines are intimately involved in the disease process. In particular, the significance of TNF α in RA has been well proven by the clinical efficacy of TNF α blockade in patients with active disease (Elliott, et al. 1993; Moreland, et al. 1997). The activation of blood monocytes before entry into the joint in RA has previously been documented. PB monocytes from RA patients, compared with monocytes from healthy individuals, express higher levels of adhesion molecules (Liote, et al. 1996; Shinohara, et al. 1992) and show increased phagocytic activity (Steven, et al. 1984). In addition, RA monocytes are able to spontaneously produce inflammatory molecules, including cytokines such as TNFα, IL-1, and IL-6 (Shore, et al. 1986; Goto, et al. 1990; Stuhlmuller, et al. 2000), the arachidonic acid metabolites (Seitz, et al. 1982), complement components (Ceulaer, et al. 1980), and angiotensin-converting enzyme (Goto, et al. 1990). Furthermore, both quantitative and qualitative alterations of myelomonocytic lineage cells in the bone marrow have been reported (Ochi, et al. 1988; Hirohata, et al. 1995; Tomita, et al. 1997). These findings strongly suggest that the monocyte/macrophage system is activated in RA. Macrophages are principal drivers of synovial inflammation in

RA, a prototype immune-mediated inflammatory disease. Monocytes/macrophages accumulate in synovium, where they play a central role in inflammation and joint destruction. Interleukin (IL) and tumour necrosis factor (TNF) alpha are pleiotropic cytokines produced predominantly by macrophages which have been implicated in the pathogenesis of RA. In human peripheral blood, two monocyte subpopulations with distinct functional properties have been defined by their expression of CD14 and CD16 molecules. The cytokine macrophage migration inhibitory factor (MIF), CD14, and Toll-like receptor 4 (TLR4) are interlinked components with clearly defined roles in immunologic and inflammatory pathways (Calandra, 2003). MIF regulates innate immune responses through the modulation of pattern recognition receptors like TLR4 (Roger, et al. 2001). TLR4, a signal-transducing receptor, is mainly responsible for the recognition of bacterial lipopolysaccharide. In brief, TLR4 specifically associates with lymphocyte antigen 96 (MD2) and, together with CD14, forms a lipopolysaccharide complex that aides in lipopolysaccharide recognition and nuclear factor NFκB activation (Calvano, et al. 2003). So the present study is designed to investigate the expression of CD14, CD16, TLR4 and MIF in blood monocytes in RA.

Materials & Methods

Study population and sampling

The study population consisted of 527subjects, 257 with active RA and 270 healthy controls. The RA group was composed of only patients with a documented history and having a current episode, whereas the healthy control group included subjects without any history of RA or any other autoimmune disorder. Written and signed informed consent was obtained from all patients. The study protocol and procedures were approved by the Institutional Ethics Committee, Deccan College of Medical Sciences (Hyderabad, India).

A total of 6 mL peripheral venous blood (2 mL in plane tube for serum separation and 3 mL in heparinized vacutainer for mononuclear cells isolation) was collected. Mononuclear cells were isolated using Histopaque (Ficoll-paque) solution according to the manufacturer's instructions.

Flow cytometric analysis

Expression levels of CD14, CD16, TLR4 and intracellular MIF

Purified mononuclear cells were diluted with phosphate buffered saline (pH 7.0) at 5 x 10^6 cells/mL (containing 10–25% monocytes). Specimen processing and antibody staining were performed according to the manufacturer's instructions. For CD14 levels, PE-conjugated antibodies against CD14 (BD Biosciences, San Jose, CA) were incubated with peripheral blood mononuclear cells (PBMCs). For TLR4, PBMCs were incubated with a PE-conjugated antihuman TLR4 (clone HTA125) or mouse immunoglobulin G2a as isotype control (eBioscience, San Diego, CA). For the detection of intracellular TLR4 levels, cells were permeabilized with permeabilization buffer (eBioscience) and stained with antihuman TLR4 antibody or relevant isotype. For CD16 levels, FITC-conjugated antibodies against CD16 (BD Pharmingen, San Jose, CA) were incubated with PBMCs. Similarly, intracellular MIF expression was evaluated using anti-MIF monoclonal antibody (mAb; R&D Systems, Minneapolis, MN). In brief, freshly isolated PBMCs were incubated with anti-MIF mAb and secondary mAb conjugated with FITC. Data acquisition and analysis were performed using CellQuest software on a BD Calibur flow cytometer (BD Biosciences) that automatically and simultaneously measured the fluorescence of individual cells identified by their size-dependent light-scattering properties.

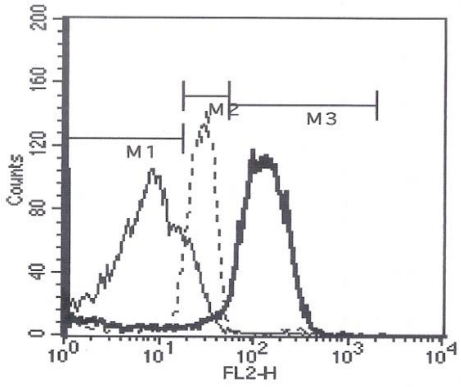
Statistical analysis

Expression of CD14, TLR4, CD16 and MIF were analyzed using one-way analysis of variance followed by Newman–Keuls multiple comparison test to determine the significance and difference between the various groups. Analysis was performed using GraphPad Prism software (version V, GraphPad software, Inc., CA).

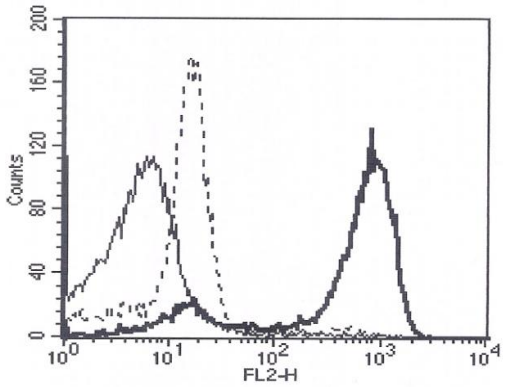
Results

Expression of CD14, TLR4, CD16 and intracellular MIF was measured in terms of mean fluorescent intensity (MFI). In CD14 expression MFI demonstrated a significant variation between RA subjects and healthy subjects ($p<0.05$; patients vs controls). A significant difference in MFI was also recorded between RA patients and healthy controls in TLR4 expression. The highest levels of CD16 expression were observed in subjects with RA when compared with healthy subjects. MIF expression levels indicated a significant difference between patients and healthy controls.

Figure 1: Histogram plot illustrating the differential expression of CD14 rheumatoid arthritis patients and healthy controls



Representative histogram plot showing CD14 expression levels in healthy volunteers



Representative histogram plot showing CD14 exression levels in RA subjects

Figure 2: CD14 expression levels differed in rheumatoid arthritis patients compared with control subjects. ***p 0.005*

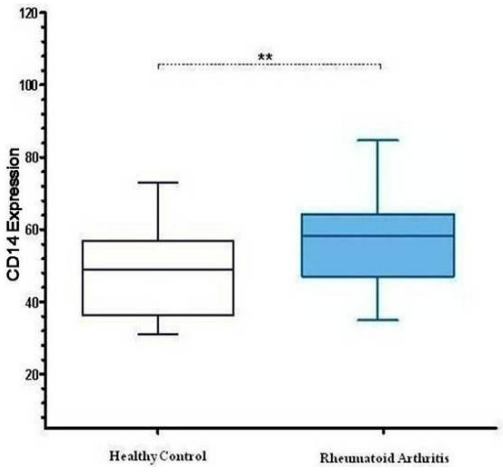


Figure 3: Histogram plot illustrating the differential expression of CD16 rheumatoid arthritis patients and healthy controls

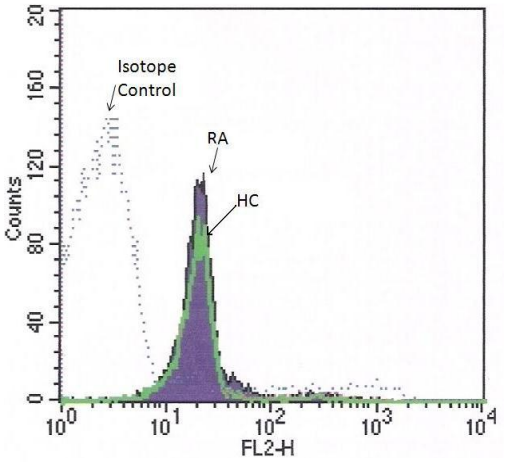


Figure 4 CD16 expression levels differed in rheumatoid arthritis patients compared with control subjects. ***p 0.005*

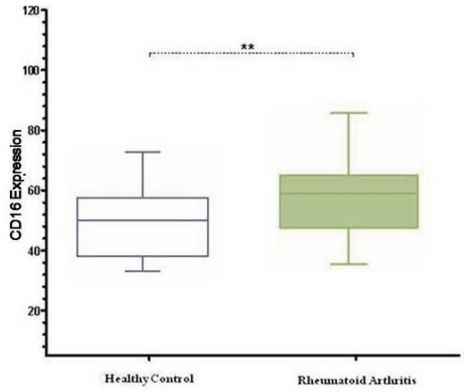


Figure 5: Histogram plot illustrating the differential expression of TLR4 rheumatoid arthritis patients and healthy controls

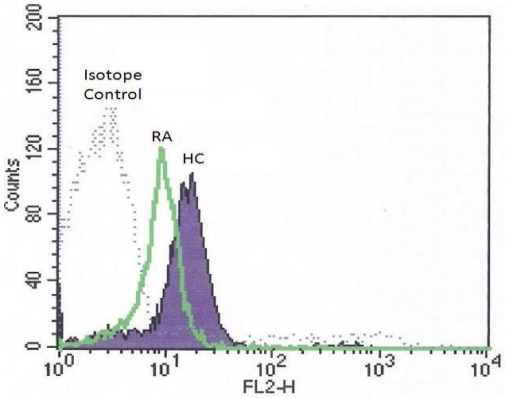


Figure 6: TLR4 expression levels differed in rheumatoid arthritis patients compared with control subjects.
***p 0.005*

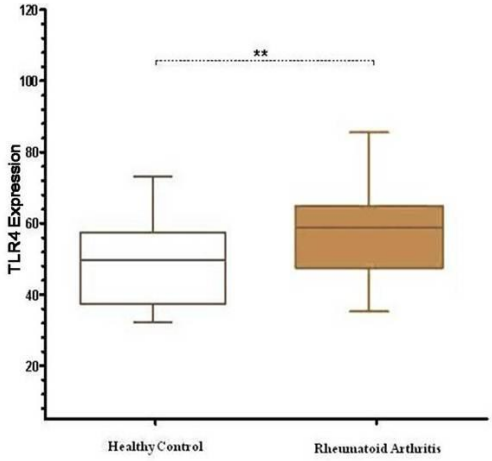
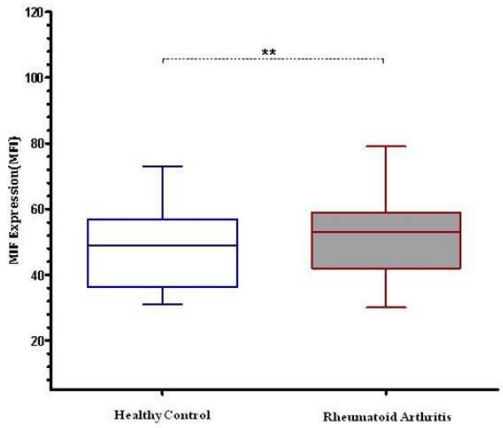


Figure 7: Intercellular MIF expression levels differed in rheumatoid arthritis patients compared with control subjects.
***p 0.005*



Discussion

CD14+, CD16+ monocytes are a subpopulation of cells that while in the circulation, has acquired features in common with mature tissue macrophages.CD16+ monocytes can rapidly migrate to the site of inflammation, where they readily mature into proinflammatory macrophages. CD14, TLR4, and MIF genes represent important and intertwined determinants of immunity. A well-orchestrated and synchronized interaction between these is highly essential for an optimal first line of defense (Admetlla, et al. 2008). Alteration in this normal process significantly dislodges the immunity network, resulting in immunologic imbalance. RA is one such paradigm of immune dysregulationas a result of persistent inflammation. We studied the effects of CD14, CD16, TLR4, and MIF on respective mononuclear cells expression levels in patients with RA. In recent years, there has been large influx of expression studies on different genes that are either directly or indirectly involved in the disease process. Because MIF, CD14, and TLR4 genes represent a triad that regulates innate immunity; the present study attempted to demonstrate the significance of expression in candidate genes (CD14, CD16, MIF and TLR4) of innate immunity in increasing the risk of RA. This study demonstrated the effect expression patterns of their respective proteins. As can be clearly seen from the data the expression of CD14 and CD 16 differed significantly between patients and controls (Fig 1, 2 and 3, 4).A similar trend was observed with TLR4 expression levels (Fig 5 and6). Kawanaka *et al* (Kawanaka, et al. 2000) suggested that the numbers of CD14+, CD16+ blood monocytes increase during the active phase of RA which supports our data. An important role for the CD16 molecule induced on monocytes in RA has recently been indicated by Abrahams *et al* (Abrahams, et al. 2000). Since MIF was first identified in 1966, its influence on the pathogenesis of many diseases, including RA, has been investigated extensively (Bloom, et al. 1996). Many studies have shown that MIF plays a pro-inflammatory role in many inflammatory diseases, such as sepsis (Bernhagen, et al. 1993), RA (Leech, et al. 1998), delayed-type hypersensitivity (Bernhagen, et al. 1996), glomerulonephritis (Lan, et al. 1997), and various tumors (Nishihira, et al. 2003). In our study a similar trend was observed with MIF expression levels (Fig 7).The link between increased TLR4 surface expression and MIF cytokine production is compelling since several lines of evidence are emerging that suggest a regulatory relationship between the two.In conclusion, this study demonstrated that the CD14, CD16, TLR4 and MIF gene modulate their expression levels on monocytes and also confer a greater risk of developing RA. Because these genes are interlinked with each other, further studies are required to gain insight how each of the genes alter the protein structure and interaction, resulting in perturbation of the delicate immunologic balance in RA.

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Conflict of interest: None

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