EVALUATION OF THE CLASSICAL COMPLEMENT PATHWAY IN ACTIVATION OF RhEUMATOID ARTHRITIS

Thesis

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Abstract

Background: Complement activation has been implicated in the pathogenesis of RA. C1q–C4 complexes are a specific parameter of classical pathway activation.

Objective: we aimed to investigate plasma levels of complement parameter; C1q & activated C4 in Rheumatoid arthritis, and also to assess their association with disease activity.

Methods: 50 RA patients classified into inactive RA group whose DAS 28 <2.6 (n=23/50) and active group had DAS 28 ≥ 2.6 (n =27/50) and control group (n=20). RA patients were submitted to full history taking, full clinical examination, routine laboratory investigations,assessment of disease activity. CIq antibodies and C4d antibodies were measured using Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Results: The median plasma levels of C1q and C4d in RA patients (9.3, 8.7 ug/ml, respectively) was statistically significant higher than the control group (2.4 and 4.8 ugEq/ml, respectively) (P=0.01, 0.001 respectively). Active RA patients had elevated levels of C1q and C4d comparing to those with inactive disease. RA patients with elevated median levels of C1q & C4d had higher statistically significant difference than those with normal levels regarding duration of morning stiffness, VAS, 28 TJC, 28 SJC, DAS28, ESR and CRP. There was a significant positive correlation between the median of C1q and C4d levels in our RA patients (r= 0.39, P = 0.004). Also these complement parameters had positive correlation with duration of morning stiffness (r= 0.539,0.275, P = 0.001,0.05 , respectively), VAS (r=0.461, 0.335,P=0.001,0.017), DAS-28 (r = 0.523,0.393; P=0.001), ESR (r = 0.561,0.454; P= 0.001) and CRP (r =0.492, 0.411; P= 0.001).

Conclusion: we can conclude that the classical complement pathway activation is correlated with disease activity in RA patients, supporting this assumption C1q and C4d levels were found to be significantly correlated with disease activity parameters.

Key words: rheumatoid arthritis, disease activity parameters, classical complement pathway, C1q, C4d.

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List of Abbreviations

ACPA	Anti-c**i**trullinated protein antibodies
ACR	American College of Rheumatology
ADAMTS	A Disintegrin and metalloproteinase with thrombospondin motif
ANCA	Anti-neutrophil cytoplasmic antibody
ANOVA	ANalysis Of VAriance
APCs	Antigen-presenting cells
ATP	Adenosine triphosphate
C`	Complement
CCL	Chemokine (C-C motif) ligand
CD28	Cluster of Differentiation 28
CH50	Complement hemolytic
CIA	Collagen-induced arthritis
CRP	C–reactive protein
CTLs	**C**ytotoxic T cells
CTLA4	Cytotoxic T cell-associated antigen 4
CXCL13	Chemokine (C-X-C Motif) Ligand 13
DAF	Decay-accelerating factor
DAS-28	Disease activity score in 28 joints
DC	Dendritic cells
EA	Sheep erythrocytes
EBV	Epstein–Barr virus
E-C4d	Erythrocyte-bound C4d
ECs	Endothelial cells
EDTA	Ethylene diamine tetraacetic acid

EGF	Epidermal growth factor
EGTA	Ethylene glycol tetraacetic acid
ELISA	The enzyme-linked immunosorbent assay
EULAR	The European League Against Rheumatism
FADD	Fas-associated via death domain
Fc	Fragment crystallizable
FcγR	The Fc receptors for IgG
FD	Factor D
FLIP	FLICE-like inhibitory protein
FLSs	Fibroblast-like synoviocytes
GH	General health
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GpE	Guinea pig erythrocytes
HLA	Human leukocyte antigen
HLA-DRB1	Human leukocyte antigen, class II, DR beta 1
HUVS	Hypocomplementemic urticarial vasculitis syndrome
IFN-γ	Interferon-γ
Ig G Fc	Immunoglobulin G ,Fragment, crystallizable
IL-1Ra	IL-1 receptor antagonist
IL	Interleukin
LT-β	Lymphotoxin- β
MAC	Membrane attack complex
MAps	MBL-associated proteins
MASP	Mannan-associated serine protease
MBL	Mannan-binding lectin

MHC	Major histocompatibility complex
MMP	Matrix metalloproteinases
Murin BM-DC	Murine bone marrow-derived DCs
NK	Natural killer cells
OPG	Osteoprotegerin
OR	Odds ratio
PADI4	Peptidyl arginine deiminase 4
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
PTPN-22	Protein tyrosine phosphatase, non-receptor type- 22
RA	Rheumatoid Arthritis
RANKL	Receptor Activator for Nuclear Factor κ B Ligand
RbE	Rabbit erythrocytes
SAP	Serum amyloid P
SCR-p	Short consensus repeats protein
SE	Shared-epitope
SERPINS	Serine proteinase inhibitors
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SNPs	Single-nucleotide polymorphisms serine proteinase inhibitors
SPSS	Statistical Package for the Social Sciences
T reg cells	Regulatory T cells
TCC	Terminal complement complex
TCR	T cell receptor
TGF-ß	Transforming growth factor-β
TIE2	Tyrosine kinase with immunoglogulin-like loops

TIMP	Tissue inhibitor of metalloproteinase
Th	T helper
TNF-α	Tumor-necrosis factor-α
Tr cells	suppressor T cells
TRAIL	TNF-related apoptosis inducing ligand
TRIFMA	Time-resolved immunofluorometric assay
VAS	Visual analogue scale
VEGF	Vascular endothelial growth factor
VLA	Very late activation antigen
WBCs	White blood cells
28 SJCs	28 swollen joints count
28 TJCs	28 tender joints count

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterized by chronic inflammation of the joints; eventually leading to bone and cartilage destruction. Complement activation has been implicated in the pathogenesis of the disease. Various studies identify complement activation as a main event in the inflammatory cascade in RA ***(Makinde et al., 1989 and Hietala et al., 2004).***

Involvement of complement in the pathogenesis of RA was also confirmed in experimental studies.Collagen-induced arthritis (CIA), an experimental animal model for human RA, was induced in C3- and factor B–deficient mice. In complement-deficient mice, arthritis was reduced or completely absent, whereas normal mice were susceptible to CIA, indicating an important role of complement in the induction of disease ***(Hietala et al., 2002).***

The complement system is one of the major effector mechanisms of the innate immune system and it plays an important role in immune defence. The biological functions of complement are opsonization and phagocytosis, stimulation of inflammatory reactions **(*Abbas et al., 2000).***

The complement system is formed by an enzymatic cascade composed of plasma proteins that, once stimulated, can achieve tremendous amplification and effectively fight the invading microorganisms. Under certain conditions, a pathological immune reaction may develop against complement components, which on one hand reflects an underlying

autoimmune process and may lead to inflammation and tissue injury. ***(Potlukova and Kralikova, 2008).***

C1q, C4 complexes are a specific parameter of classical pathway activation; increased plasma levels of these complexes in RA indicate involvement of the classical pathway of complement in RA ***(Wouters et al., 2006).***

Levels of the acute-phase protein, C-reactive protein (CRP), are elevated in the majority of patients with RA and are associated with disease activity. CRP bound to a ligand can activate complement, and there is evidence that CRP-mediated activation of complement occurs in RA. Both immune complexes and ligand-bound CRP activate complement via the classical pathway ***(Romero et al., 1998 and Molenaar et al., 2001).***

Aim of work

This study will be performed to investigate plasma levels of complement parameter, and also to assess the association of these parameters with disease activity.

Rheumatoid Arthritis

Introduction

The disease that is currently clinically recognized as rheumatoid arthritis (RA) was first defined by Sir Alfred Baring Garrod in 1859, when he differentiated it from gout, which we now know is caused by crystals of monosodium urate**.** In the 1870s, there was uncertainty among physicians whether acute rheumatism (rheumatic fever, RF) and chronic rheumatism (rheumatoid arthritis, RA) were separate diseases or that there was one "rheumatism" whose manifestations differed due to influences of age, heredity, and environment (***Klareskog et al.,2006).***

Rheumatoid arthritis is a systemic autoimmune disease which is characterized by chronic inflammation of the joints resulting in joint destruction. Patients experience chronic pain and suffering, functional impairment, increasing disability, without treatment there is a reduction in life expectancy. The availability of effective therapies makes it imperative to identify patients early so that control of inflammation can prevent joint destruction and disability ***(Mody and Cardiel, 2008).***

RA has a significant impact on health-related quality of life; it is associated with increased health-care costs and an increased mortality when affected patients are compared with the general population (***Sihvonen et al., 2004).***

Epidemiology

The incidence of RA varies across populations. Estimates from North America and Northern Europe range from 20 to 50 cases per 100,000