Impaired expression of erythrocyte glycosyl-phosphatidylinositol-anchored membrane CD59 in patients with psoriatic arthritis. Relation to terminal complement pathway activation


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ABSTRACT

Objective. Complement-mediated injury is regulated by many factors; among these CD59 has been identified as a widely distributed glycoprotein that inhibits membrane C5b-9 (terminal complement component) formation. The aim of the study was to assess erythrocyte CD59 expression in patients with psoriatic arthritis in order to understand the role of CD59 in the pathogenesis.

Methods. Washed erythrocytes from 50 patients with psoriatic arthritis, 8 with cutaneous psoriasis and 24 healthy subjects were incubated with monoclonal anti-CD59 antibody followed by a second FITC-conjugated antibody and fluorescence intensity analysed by FACScan flow cytometer to assess their CD59 membrane expression. SC5b-9 levels were measured in the plasma by ELISA and results compared with CD59 values. Immune complexes, complement C3 and C4 and rheumatoid factor were also determined.

Results. Impaired expression of erythrocyte membrane-anchored CD59 was found in patients with psoriatic arthritis; the lowest levels were seen in active patients (p < 0.01). Increased SC5b-9 was seen in the plasma of patients with active disease. An inverse correlation was also found between plasma SC5b-9 and the CD59 expression levels (r = -0.81, p < 0.001).

Conclusion. The low CD59 expression on erythrocytes from patients with psoriatic arthritis may be an index of a low tissue CD59 expression. This impairment could facilitate the activation of complement pathway and increase the risk for arthritis. Membrane attack complex formation in deficient membrane bound CD59 may also exacerbate synovial cell injury and inflammation.

Introduction

Psoriatic arthritis (PsA) is an inflammatory arthritis which occurs in 8-10% of patients with psoriasis (1). Knowledge of the mechanism responsible for damage of the articular tissue, cartilage and bone in inflammation arthritides such as PsA is limited (2). Activation of the complement system is implicated in a wide range of immunological and non-immunological diseases. Complement activation occurs in psoriasis (3). The complement system is also thought to play an important role in the recruitment of neutrophils within the epidermis and in psoriatic lesional skin. It can also activate synoviocytes. Activation of the complement system results in the production of biologically active molecules culminating in the generation of a cytolytic C5b-9 complex on target membranes and of a non-cytolytic fluid-phase (SC5-9) complex that may be detected in the plasma (4). Complement-mediated destruction of autologous cells is regulated by many factors; among these CD59 (membrane inhibitor of reactive lysis) has been identified as a glycoprotein widely distributed in tissues that inhibits the transmembrane channel-forming function of homologous C8 and C9 at the final stage MAC formation (6). CD59 belongs to a family of membrane proteins that are attached to the outer leaflet of the cell membrane through a glycosyl-phosphatidylinositol (GPI) moiety (7). It has been recently demonstrated that GPI-anchored proteins are constitutively down-regulated in psoriatic skin (8). Erythrocytes of many patients with arthritis display a relative deficiency of complement receptor 1 (CR1) with impaired capacity to clear immune complexes (9). CD59 is also expressed on erythrocytes where it is able to prevent MAC formation and lysis (10).

CD59 expression on erythrocytes of patients with psoriatic arthritis and the role of complement-regulatory proteins in the pathogenesis of this disease require investigation. The present study was conducted to study the role of CD59 in the pathogenesis of PsA. In this paper we report the level of expression of CD59 on erythrocytes of patients with PsA and its correlation with the SC5b-9 measured in the plasma of these patients. Finally, these results were correlated with the disease activity.

Patients and methods

Patients

Fifty adult patients (28 males and 22 females, mean age 47 ± 15 years, range 20-75) who had moderate to severe...
plaque psoriasis and arthritis (11) and who were negative for rheumatoid factor were included in the study. Twenty-two patients were classified as having asymmetrical oligoarthritis (<4 localisation), 28 as having polyarthritis (2 with and 26 without axial involvement). Twenty-two of the patients (6 with oligoarthritis and 16 with polyarthritis included patients with spondylitis) had active disease. Criteria for activity were at least 3 swollen and tender joints, morning stiffness of at least 30 min. duration, pain on a visual analog scale (VAS) > 20 mm, the patient’s global assessment of disease activity ≥ 2 on a 5-point ordinal scale, and abnormal ESR and CRP levels. The normal controls consisted of volunteers and laboratory personnel with no history of rheumatic and/or psoriatic disease; 16 were males and 8 females ranging in age between 23 and 62 years (mean 43 ± 11 yrs.). Eight patients (5 males, 3 females) with cutaneous psoriasis but no arthritis were also included in the study as disease controls. The diagnosis of psoriasis in both arthritic and non-arthritic patients was confirmed by a dermatologist. The study was reviewed by the ethics committee of our department and all patients gave their consent to participate after they were informed of the nature and purpose of the study.

**Cell preparation**

Venous blood was collected in EDTA. After separation of plasma by centrifugation at 800 g per 10 minutes at 4°C, erythrocytes were washed three times with PBS (0.1 M, pH 7.4) containing 1% BSA and cells resuspended in the same buffer.

**Measurement of CD59 erythrocyte expression by flow cytometric analysis**

The method used was similar to that recently described for CD59 measurement on isolated vein endothelial cells (12). Washed erythrocytes were resuspended at 2 x 10⁶ cell/ml and a 300 µl cell suspension was reacted with the first (unconjugated) monoclonal (mo-Ab) antibody (anti-CD59 10 µg/ml; ICN Biomedicals, Irvine, CA) on ice for 20 minutes, washed three times with PBS-BSA and incubated for an additional 20 minutes with the second antibody (FITC-conjugated F(ab)₂ goat anti-mouse IgG. Isotype control IgG was used as a negative control. The stained cells were washed twice and then analysed by FACScan flow cytometer (Becton Dickinson, Mountain View, Calif., USA). All measurements were carried out in duplicate and the results were expressed as mean fluorescence intensity (MFI). To study the possible effect of autoantibodies and immune complexes on CD59 detection, normal erythrocytes were pre-incubated with patient sera and CD59 expression was measured as above. No signs of haemolysis were observed during the washing procedures. Soluble CD59 was also measured by ELISA (12) in synovial fluid obtained from two of the patients.

**Results**

To study the role of CD59 on PsA, the expression of CD59 was studied on the erythrocytes of normal and PsA subjects. In PsA patients the expression of CD59 was significantly reduced in comparison with the expression of healthy subjects. In PsA patients, the mean MFI was 60.4 ± 5.3, while in healthy individuals it was 71.7 ± 5.9 (p < 0.01). MFI levels were found to be 55.8 ± 4.07 in patients with active disease and 63.6 ± 6.56 in inactive patients (p < 0.01) (Fig. 1). Levels similar to those found in inactive patients were observed in subjects with cutaneous psoriasis without arthritis (64.7 ± 2.9). Although MFI levels were found to be

**Statistics**

Results were expressed as the mean ± SD. Results were compared by the Student t-test for unpaired data. Correlations were calculated with Spearman’s correlation coefficient. P values < 0.05 were considered significant.

**Fig. 1.** CD59 membrane expression of erythrocytes from patients with active or inactive psoriatic arthritis and from healthy subjects and patients with cutaneous psoriasis only. MFI: mean fluorescence intensity.
Table I. CD59 expression on erythrocyte (E) membranes from 3 normal subjects (N) after incubation with sera from 3 different patients with active psoriatic arthritis (S).

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BCD59 expression on untreated erythrocytes. Results are expressed as mean fluorescence intensity.

CD59 expression on erythrocytes in PsA / G. Triolo et al. found in the synovial fluid of patients with rheumatoid arthritis (13). ICs were detected in a small number of both active and inactive patients at low levels (not shown). Levels of C3 and C4 fragments were not significantly different between patients and healthy controls, nor were they comparable with the other variables (not shown). No relationship was found between IC levels and SC5b-9 or CD59 levels (not shown).

Discussion
The complement system is constantly activated in vivo and could induce damage to autologous cells including epidermal and synovial cells. Recent functional, structural and genetic studies have demonstrated the existence of several molecules embedded in the cell membranes that inhibit complement activation on the cell surface and thereby protect them from autologous complement (15). The formation of membrane attack complex (MAC) on self cells is strictly regulated by a recently described 18,000-20,000 KDa glycoprotein (membrane inhibitor of reactive lysis, CD59) (16). In particular, CD59 was identified as a glycoprotein that is widely distributed in the tissues and inhibits the transmembrane channel-forming function of homologous C8 and C9 at the final stage of MAC formation (6). CD59, together with other molecules, belongs to a family of membrane proteins that are attached to the outer leaflet of the cell membranes through a GPI (glycosyl-phosphatidylinositol) moiety (17). CD59 can be removed from cell surfaces by PI-PLC treatment (18). This is in agreement with the finding that CD59 (and CD55) expression is absent from the surface of affected erythrocytes in patients suffering from paroxysmal nocturnal haemoglobinuria, where glycolipids are not expressed on their surface (19). Hyperproliferation of keratinocytes in psoriasis has been found to be associated with excessive activation of the phospholipase C (PLC)/protein kinase (PKC) signal transduction system (20). It was also observed that GIP-specific PLC was activated in the membrane of psoriatic epidermal cells, rendering these cells devoid of proteins which are anchored to the cell membrane through their GPI moiety. The expression of GPI proteins was found to be decreased in non-lesional psoriatic skin and virtually abolished in lesional psoriatic skin (8).

There is evidence that the complement system participates in the pathogenesis of rheumatoid arthritis (RA) (21). Reduced complement-regulatory protein expression and activation of complement cascade on erythrocyte from patients with RA was also more recently demonstrated (22).

To elucidate the role of regulatory complement protein, i.e CD59, in the pathogenesis of psoriatic arthritis, we evaluated the levels of CD59 on erythrocytes from PsA patients and their relationship with the plasma levels of the terminal complement components (SC5b-9). We considered in fact the
erythrocyte CD59 levels as representative of the CD59 tissue distribution in psoriasis. Abnormal CD59 erythrocyte expression was found in psoriatic patients in comparison to normal subjects, the lowest expression being seen in the patients with arthritis. There are still no clinical examples of altered CD59 expression on erythrocytes from PsA patients, nor studies indicating the failure of erythrocyte CD59 to act as a down-regulator of complement activation. Our present findings, although not specifically addressing the question, show an inverse correlation between erythrocyte CD59 and SC5b-9 levels which could indicate a role of CD59 in the control of complement activation in PsA. It is still an open question whether low CD59 levels may be the cause or only indicate a risk for arthritis in psoriasis. Recently an acute arthritis model in rats triggered by functional suppression of the membrane complement regulator CD59 in knee joints was described (23), as well as the effects of systemic and intra-articular inhibition of complement on progression of arthritis in a model in which the injury was induced by blocking CD59 (24). Moreover, as previously stated, low CD59 levels were found in patients with active rheumatoid arthritis (22), suggesting a role of complement activation regulatory molecules in different types of arthritis. In conclusion, psoriasis is associated with low CD59 in both plaques and unaffected tissue and, according to the present study, with a decrease in CD59 erythrocyte expression. This lack of expression seems to be due to a metabolic defect leading to GPI hydrolysis and consequently to a loss of membrane-bound CD59. Low CD59 may in turn favour complement activation at membrane sites as demonstrated in various conditions (15) and recently by us in in vitro studies (12). The loss of membrane regulators of complement activation in psoriasis could facilitate the activation of complement pathway and increase the risk for arthritis in such patients or exacerbate synovial cell injury and inflammation.

References