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

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This work was supported by a grant from the Ministero della Università e della Ricerca Scientifica e Tecnologica of Italy. Dr. Antonina Accardo-Paumbo is a PhD student and recipient of a post-doctoral fellowship.

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Received on February 19, 2002; accepted in revised form on November 11, 2002.

Key words: Erythrocyte CD59, SC5b-9, psoriasis, arthritis.

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 C5b-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-phosphatydilinositol (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 10^6 cell/ml and a 300 µl cell suspension was reacted with the first (unconjugated) monoclonal (moAb) 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.

Measurement of plasma SC5b-9

SC5b-9 was measured by ELISA in a procedure based on monoclonal and polyclonal antibodies against a neoantigen of the complex, as recently described (13). Results were expressed as arbitrary units (AU)/ml. The normal range, defined as a 2.5 – 97.5% interval of the values obtained from 87 healthy subjects, was 3.5 – 12 AU/ml.

Other laboratory variables

C3 and C4 complement fragments were measured by standard laboratory procedures. Circulating immune complexes (ICs) were determined by the use of an anti-C3 enzyme immunoassay (14).

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.

Results

Quantification of CD59

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

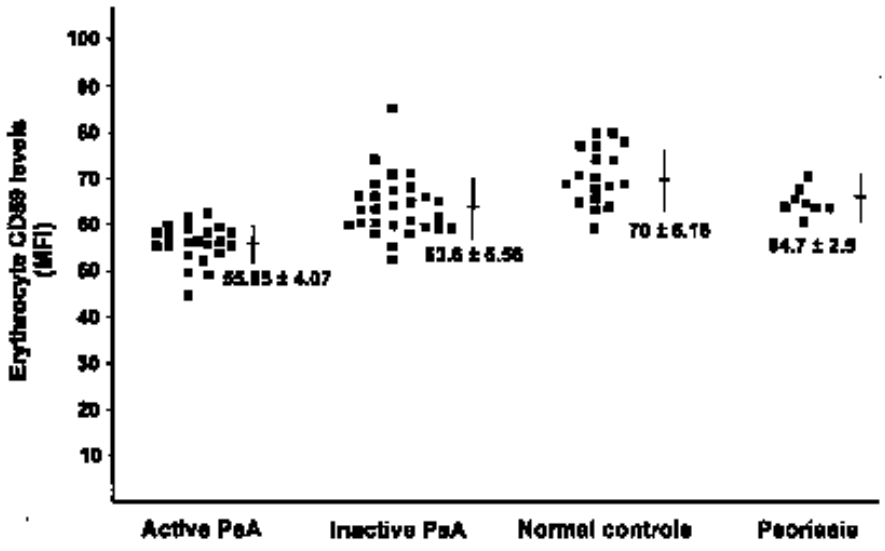

Fig. 1. CD59 membrane expression of erythrocytes from patients with active or inactive psoriatic arthritis and from healthy subjects and patients with cutaneous psoriasis only. MFI:mean fluorescence intensity.

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).

E	BL	S1	S2	S3
N1	71	74	70	68
N2	80	80	80	75
N3	77	73	79	75

BL:CD59 expression on untreated erythrocytes. Results are expressed as mean fluorescence intensity.

slightly lower in patients with polyarthritis than in patients with oligoarthritis, the difference did not reach statistical significance (not shown). No relationship was found either between MFI levels and the type of treatment, the duration of disease or the extent of skin involvement. The amounts of CD59 on normal erythrocytes was not modified by pre-incubation of normal cells with patient sera (Table I). High levels of soluble CD59 were found in synovial fluids (OD case 1: 0.340; OD case 2: 0.590; OD saline: 0.045 ± 0.012).

SC5b-9 and ICs measurement

Plasma levels of SC5b-9 were 18.8 ± 5.4 AU/ml in active PsA patients, 6.2 ± 3.1 AU/ml in inactive PsA patients and 5.4 ± 3.8 AU/ml in healthy subjects (Fig. 2). A significant inverse correlation was found between SC5b-9 levels and erythrocyte surface CD59 levels in patients with active arthritis ($r = -0.80$; $n = 22$, $p < 0.01$) (Fig. 3). High levels of SC5b-9 were found in the synovial fluid (case 1: 98 AU/ml; case 2: 84 AU/ml), which were comparable to those 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

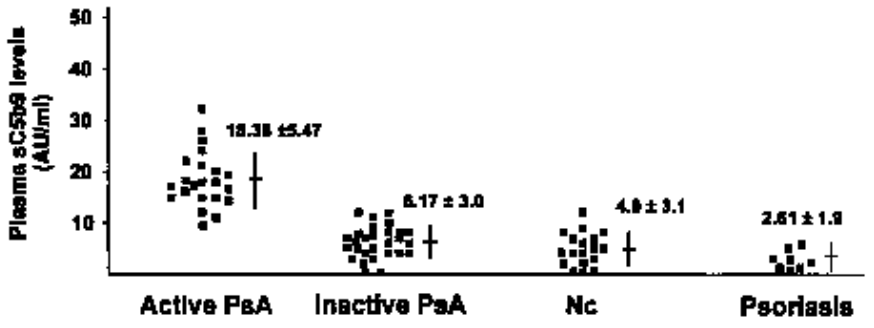

Fig. 2. SC5b-9 levels in the plasma from patients with active or inactive psoriatic arthritis and from healthy subjects and patients with cutaneous psoriasis only. AU/ml: arbitrary Units/ml.

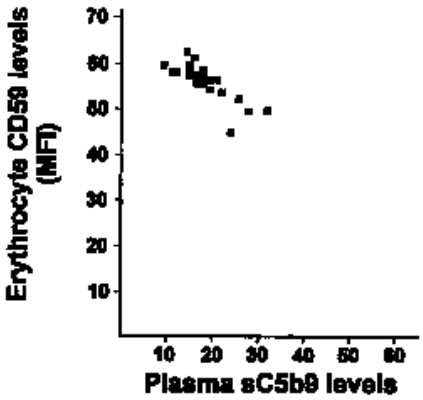

Fig. 3. Correlation between erythrocyte CD59 expression and plasma SC5b-9 levels in patients with active psoriatic arthritis.
MFI: mean fluorescence intensity; AU/ml: arbitrary units/ml; $r = -0.81$ ($n = 22$), $p < 0.001$

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 GPI-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

1. FITZGERALD O, KANE D: Clinical, immunopathogenic and therapeutic aspects of psoriatic arthritis. *Curr Opin Rheumatol* 1997; 9: 295-301.
2. POOLE AR: Immunochemical markers of joint inflammation, skeletal damage and repair: Where are we now? *Ann Rheum Dis* 1994; 53: 3-5.
3. ROSENBERG EW, NOAH PW, WYATT RJ, JONES RM, KOLB WP: Complement activation in psoriasis. *Clin Exp Dermatol* 1990; 15: 16-20.
4. DALMASSO AP, FALK RJ, RAJI L: The pathobiology of the terminal complement complexes. *Complement Inflamm* 1989; 6: 36-48.
5. TAKEMASU H, TAGAMI H: Generation of terminal complement complexes in psoriatic lesional skin. *Dermatology* 1992; 185: 246-50.
6. ROLLINS SA, ZHAO JJ, NINOMJA H, SIMS PJ: Inhibition of homologous complement by CD59 is mediated by a special selective recognition conferred through binding to C8 within C5b-8 or C9 within C5b-9. *J Immunol* 1991; 146: 2345-51.
7. NALSH LA, TONE M, THIRU S, WALMANN H: CD59 antigen: A multifunctional molecule. *Tissue Antigens* 1992; 40: 213-20.
8. VENNEKER GT, DAS PK, MEINARDI MM, MARLE J, VAN VEEN HA, BOS ASGHAR SS: Glycosylphospatydilinositol (GPI)-anchored membrane proteins are constitutively down-regulated in psoriatic skin. *J Pathol* 1994; 172: 189-97.
9. KUMAR A, MALAVIYA AN, SRIVASTAVA LM: Lowered expression of C3b receptor on erythrocytes of rheumatoid arthritis. *Immunobiol* 1994; 191: 9-20.
10. HOLGUIN MH, FREDRICK LR, BERNSHAW NJ *et al*.: Isolation and characteristic of membrane protein from normal human erythrocytes that inhibits the reactive lysis of the erythrocyte of paroxysmal nocturnal hemoglobinuria. *J Clin Invest* 1989; 84: 7-17.
11. MOLL JM, WRIGHT V: Psoriatic arthritis. *Semin Arthritis Rheum* 1973; 3: 55-78.
12. ACCARDO-PALUMBO A, TRIOLO G *et al*.: Glucose-induced loss of glycosyl-phosphatidylinositol-anchored membrane regulators of complement activation (CD59, CD55) by *in vitro* cultured human umbilical vein endothelial cells. *Diabetologia* 2000; 43: 1039-47.
13. ACCARDO-PALUMBO A, TRIOLO G, CASIGLIA D, SALLÌ L, GIARDINA E, TRIOLO G: Two-site ELISA for quantification of the terminal C5b-9 complement complex in plasma. Use of monoclonal and polyclonal antibodies against a neoantigen of the complex. *J Immunol Methods* 1993; 163: 169-72.
14. TRIOLO G, GIARDINA E, RINALDI A, BOMPIANI GD: IgA- and insulin-containing (C3-fixing) circulating immune complexes in diabetes mellitus. *Clin Immunol Immunopathol* 1984; 130: 169-77.
15. ASHGAR SS: Membrane regulators of complement activation and their aberrant expression in disease. *Lab Invest* 1995; 72: 254-71.
16. OKADA N, HARADA R, FUJITA T, OKADA H: A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. *Intern Immunol* 1989; 1: 205-8.
17. LOW MG, SALTIEL AR: Structural and functional roles of glycosyl-phosphatidylinositol in membranes. *Science* 1988; 239: 268-75.
18. BROOIMANS RA, VAN WIERINGEN PA, VAN ES LA, DAHA MR: Relative role of decay-accelerating factor, membrane co-factor protein and CD59 in the protection of human endothelial cells against complement mediated lysis. *Eur J Immunol* 1992; 22: 3135-40.
19. WOMTOVIAN R, PRINCE GM, MEDOF ME: The molecular basis of paroxysmal nocturnal hemoglobinuria. *Transfusion* 1993; 33: 852-73.
20. FISHER GJ, TALWAR HS, TAVAKKOL A *et al*.: Phosphoinositide-mediated signal transduction in normal and psoriatic epidermis. *J Invest Dermatol* 1990; 95: S15-17.
21. OLEESKY DA, DANIELS RH, WILLIAMS BD, AMOS N, MORGAN BP: Terminal complement complexes and C1/C1 inhibitor complexes in rheumatoid arthritis and other arthritic conditions. *Clin Exp Immunol* 1991; 84: 250-5.
22. ARORA M, KUMAR A, DAS SN, SRIVASTAVA LM: Complement-regulatory protein expression and activation of complement cascade on erythrocytes from patients with rheumatoid arthritis. *Clin Exp Immunol* 1998; 111: 102-6.
23. MIZUMO M, NISHIKAWA K, GOODFELLOW RM *et al*.: The effects of functional suppression of a membrane-bound complement regulatory protein, CD59, in the synovial tissue in rats. *Arthritis Rheum* 1997; 40: 527-33.
24. MIZUMO M, NISHIKAWA K, MORGAN BP, MATSUO S: Comparison of the suppressive effects of soluble CR1 and C5a receptor antagonist in acute arthritis induced in rats by blocking CD59. *Clin Exp Immunol* 2000; 119: 368-76.