Fc-RECEPTOR FUNCTION IN HENOCH-SCHÖNLEIN DISEASE OF CHILDHOOD

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Summary

The Fc-receptor function of circulating monocytes (CM) and/or of splenic macrophages (SM) was followed in eight children presenting with rheumatoid purpura. The Fc-receptor of CM and/or of SM was decreased in all cases during the acute phase of the disease without any correlation with the plasma values of C3, C4, C3PA or immune complexes. When a clinical improvement occurred, a reversal of the Fc-receptor blockade was noted. In contrast, the Fc-receptor function remained abnormal in three children with persistent nephritis. The sequential measurement of Fc-receptor function may be of predictive value in rheumatoid purpura of childhood.

Introduction

Rheumatoid purpura (Henoch-Schönlein purpura) is a rather common disease of childhood, most cases healing without sequelae. Prognosis is largely determined by the evolution of the associated nephropathy, which is present in 28 per cent of cases [1]. The aetiology of this disease remains unknown, but it is generally considered that immunological mechanisms are involved in the pathogenesis. This hypothesis is supported by the demonstration of different immunological abnormalities in Henoch-Schönlein disease, i.e. the presence of cryoglobulinaemia [2], of circulating immune complexes [3], the activation of the complement cascade [4], and the renal deposition of IgA, IgG, fibrin and C3 [5].

The Fc-receptor function of splenic macrophages is altered in different immune complex-mediated diseases, such as lupus nephritis and some cases of rapidly progressive glomerulonephritis [6]. Fc-receptor function may also be decreased in Henoch-Schönlein purpura, and this may be related either to renal involvement, or to different immunological parameters during the course of the disease.
Material and methods

 Patients

The Fc-receptor function of circulating monocytes (CM) and of splenic macrophages (SM) was followed in six boys and two girls (aged from 16 months to 11.5 years) suffering from rheumatoid purpura. The main clinical features are summarised in Table I. Three children presented with cutaneous lesions and arthritis only. The others exhibited renal disease; two children presented with haematuria and albuminuria during the acute phase of the disease only, whereas the others exhibited persistent haematuria and/or albuminuria several months after the acute phase. Renal function remained normal in all children.

Assessment of Fc- and C3-receptor functions of CM in vitro

Ten millilitres of blood were drawn under sterile conditions, heparinised and mixed with 30ml phosphate-buffered saline. Five millilitre aliquots of the mixture were layered on 2ml Ficoll-Hypaque (Pharmacia Fine Chemicals). The tubes were centrifuged at 1000g for 20 minutes. The mixed mononuclear cells obtained from the interface were washed three times with RPMI medium and were resuspended in the same medium containing two per cent fetal calf serum at a concentration of $1 \times 10^6$ leucocytes per millilitre; 50μl portions of this suspension were added to plastic wells in sterile flat bottom microtitre plates (Falcon, Becton, Dickinson and Co., Oxnard, California, USA) and incubated for 30 minutes at 37°C. More than 90 per cent of adherent leucocytes were monocytes, as judged by their morphologic appearance after staining for non-specific esterase and by their capacity to ingest latex beads [7]. Adherent monocytes were covered with 50μl of a 0.4 per cent suspension of sheep erythrocytes coated either with IgG anti-sheep red blood cell antibodies (for the Fc test) or with IgM anti-sheep red blood cell antibodies and human complement (for the C3 test). In some experiments, monocytes were first incubated for 30 minutes at 37°C with 100μl of RPMI medium containing 1μg/ml of trypsin (Sigma Co., St Louis, Missouri, USA) [7], before the addition of IgG-coated erythrocytes. After incubation for 20 minutes, each well was washed thoroughly with phosphate-buffered saline, dried and stained with Giemsa stain. The percentage of monocytes with Fc- or C3-receptor function was quantified in each of five microtitre wells, by scoring at least 100 cells presenting with three or more adherent monocytes or one or more ingested erythrocytes [7]. The specificity of the test was ascertained by pre-coating the wells either with monomeric human IgG (1mg/100μl during 18 hours at 4°C) or with their F(ab′)2 fragment.

Assessment of Fc-receptor function of SM in vivo

Two millilitres of heparinised blood was collected. 99Tc-labelled heat-damaged autologous red blood cells were prepared according to the method of Lockwood et al [6]. Forty minutes after intravenous injection, a gamma camera scintigraphy was performed, and the spleen to liver ratio was determined [6].
<table>
<thead>
<tr>
<th>Case number</th>
<th>Age</th>
<th>Sex</th>
<th>Onset of the disease (weeks before testing)</th>
<th>Initial symptoms</th>
<th>Follow-up (months)</th>
<th>Evolution</th>
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<td>Arthralgia</td>
<td>Renal involvement</td>
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<td>1</td>
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<td>F</td>
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<td>1 year 4 months</td>
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<td>4</td>
<td>6 years 8 months</td>
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<td>5</td>
<td>7 years</td>
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<td>8</td>
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<td>4 years 2 months</td>
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<tr>
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<tr>
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<td>11 years 6 months</td>
<td>F</td>
<td>260</td>
<td>+</td>
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</table>
Serological studies

C3, C4, C3PA plasma values were determined by laser nephelometry and immune complex plasma concentrations by conglutinin and the Clq binding assays.

Results

*Fc-receptor function of CM and SM during an acute phase of rheumatoid purpura*

The Fc-receptor function of CM was first determined in 10 normal adult volunteers. More than 60 per cent of CM were able to bind, at least, three IgG-coated erythrocytes (Figure 1). In IgG-coated plates, the percentage of normal CM ‘rosetting’ was lower than 10 per cent, but returned to a normal range (60 to 90 per cent) in plates coated with F(ab’)2 fragments of human IgG. The Fc-receptor function of CM was then measured in five children (case numbers 1, 2, 3, 4 and 6) (Table I) during an acute phase of rheumatoid purpura. In all cases, the Fc-receptor function of CM was considerably altered, the percentage of CM ‘rosetting’ with IgG-coated erythrocytes being lower than 20 per cent (Figure 1). At the same time, the C3-receptor function of CM isolated from these children remained normal, i.e. more than 60 per cent of CM were able to bind, at least, three erythrocytes coated with IgM antibody and human complement. In case number 1, prior incubation of monocytes with trypsin reversed the Fc-receptor blockade.

The Fc-receptor function of SM was first studied in 10 normal subjects. The spleen to liver uptake ratio of 99Tc-labelled, heat-damaged red cells were always higher than or equal to 10 (Figure 1). The Fc-receptor function of SM was decreased in two of three children during an acute phase of rheumatoid purpura.

![Graph](image)

Figure 1. Fc-receptor function of CM and SM in eight children presenting with rheumatoid purpura and in 10 normal volunteers. • number of patient

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Fc-receptor function of CM and of SM after an acute phase of rheumatoid purpura

The Fc-receptor binding activity of CM and of SM was measured in seven children after an acute phase of rheumatoid purpura (case numbers 1, 3, 4, 5, 6, 7 and 8) (Table I). Four of them had been tested during the acute phase (case numbers 1, 3, 4 and 6); a reversal of the Fc-receptor blockade of CM was noted in all cases (Figure 2), but the Fc-receptor function of SM remained depressed in cases 3 and 4, in which haematuria and/or albuminuria persisted.

![Graph](image)

Figure 2. Fc-receptor function of CM in four children with rheumatoid purpura during and after the acute phase

Patients 5, 7 and 8, which were not tested during the acute phase, also demonstrated Fc-receptor blockade (of CM in case 7, of SM in cases 5 and 8); cases 5 and 7 exhibited persistent urinary abnormalities months after the acute phase (Table I); patient 8 never presented urinary changes, but demonstrated 10 relapses of purpura over five years and was tested a fortnight before a purpuric rash.

**Correlation between the Fc-receptor function of CM or of SM, and different immunological parameters**

No significant correlations were observed, either between the Fc-receptor functions measured in vitro and in vivo, or between the Fc-receptor functions of CM and of SM, and the C3, C4, C3PA and immune complex plasma values obtained during and after the acute phase of the disease in the same patients (p > 0.05). However, the Fc-receptor function of SM was particularly depressed when the Clq binding activity of the sera was very high (50% of specific precipitation at least).
Discussion

The results show that the Fc-receptor function of the reticulo-endothelial system is altered in rheumatoid purpura of childhood. The Fc-receptor blockade is always obvious during the acute phase of the disease. This blockade is reversed when the patient completely heals. In contrast, the Fc-receptor function remains altered in patients with persistent haematuria and/or albuminuria, or presenting with relapsing purpura. The reasons for this Fc-receptor blockade remain unknown. Two major hypotheses might be advanced:

1. The Fc-receptors are saturated by immune complexes, leading to a competitive inhibition between the Fc-fragment of IgG present in immune complexes and that of IgG coated to erythrocytes; this is supported by the reversal of the blockade by a prior incubation of CM with trypsin;

2. The Fc-receptor function could be initially abnormal as in dermatitis herpetiformis [8], and was therefore unable to clear circulating immune complexes.

The lack of correlation between the in vitro and in vivo tests might be explained by the fact that heat-damaged autologous erythrocytes are probably not only cleared by Fc-receptors but also by C3- and/or other unknown receptors [6]. Experiments are now being developed in order to study the Fc-receptor function of SM using autologous erythrocytes coated with anti-D IgG and labelled with $^{99}$Tc.

No correlations between the in vitro and in vivo tests and the levels of circulating immune complexes have been observed. However, the Fc-receptor blockade of SM was mainly observed when the Clq binding activity of the sera was the highest. The same observation has been made in adult patients with rheumatoid arthritis [9] and lupus nephritis [10]. The persistence of an abnormal Fc-receptor function in patients with persisting nephritis or relapsing purpura lead us to believe that these tests could be of predictive value. Further information and long-term follow-up will be necessary however to confirm this hypothesis.

Acknowledgments

I particularly thank Jacqueline Mathieu, Yvonne Pirard and Aline Desourdoux for their technical assistance, and D Croughs for expert secretarial help.

References

3 Levinsky RJ, Barratt TM. Lancet 1979; ii: 1100

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Open Discussion

VALENTYN (Leiden) Do you think that clearance of heat damaged erythrocytes is really a function of Fc receptor clearance mechanisms? Are heat damaged erythrocytes really the best method of studying this? In your abstract you state that you did not find any correlation with the presence of immune complexes or complement depression. It might well be that if you look at immune complexes with the right assay, specifically looking for IgA complexes, which you did not do by using a Clq binding assay, you might find a correlation with decreased clearance activity. This has been shown previously and the same may hold true for looking at complement break down products. Most patients we have seen with Henoch-Schönlein purpura have a normal C3 but they do have elevated C3b levels during activity.

DAVIN In answer to your first question I can say that probably the use of heat damaged erythrocytes is not the most specific test for measuring Fc receptor function of splenic macrophages. It could have been better if we had used erythrocytes coated with IgG anti-D. We have performed both in vivo tests in adults presenting with rheumatoid arthritis and there was a correlation between those tests. Both tests were depressed and the ratio was lower than normal values. For your second question about immune complexes we have used the conglutinin assay and the Clq liquid phase assay but not specific assays of IgA. This is probably the reason why we have not had any correlation with those results.