Reconstitution of the alternative pathway of complement by plasma infusions given to a patient with an SLE-like syndrome associated with a hereditary C3 dysfunction

Bo Nilsson, Ulf R Nilsson, Alex Karlsson-Parra, Gunilla Sjölin-Forsberg, Roger Hägggren

Abstract

Objective—To reconstitute a dysfunctional form of complement factor C3 in a patient with a systemic lupus erythematosus (SLE)-like syndrome.

Methods—The propositus was treated with plasma infusions during five sessions over a period of eight months.

Results—The alternative pathway was reconstituted to normal levels for approximately two to three days after each infusion. C3 fragments were incorporated into previously detected deposits of IgG and IgM at the dermal-epidermal junction and the immune complex levels gradually decreased during the whole treatment period.

Conclusion—The reconstitution appears to result in the solubilisation of tissue immune complexes and a subsequent transportation to the fixed macrophage system.


The strong association between deficiencies of the complement system and the occurrence of immune complex associated inflammatory conditions such as systemic lupus erythematosus (SLE),1–4 emphasises the important role of complement in immune complex (IC) metabolism.

We recently reported of a patient with an SLE-like syndrome which was associated with a dysfunction of C3.5 The propositus suffered from fatigue, swollen fingers, arthralgia and Raynaud’s syndrome combined with the presence of anti-centromere antibodies. The dysfunction was a result of a double deficiency with a null allele originating from the father and one allele for the dysfunctional C3 from the mother. The haemolytic function by the alternative pathway (APW) was shown to be completely eliminated while the CPW function was reduced by 75%. The reason for this was that the dysfunctional C3, especially in the APW, was not cleaved into C3a and C3b due to an inability of the C3 to interact properly with the convertases.

We report the results of repeated plasma infusions into the C3 dysfunctional patient in an attempt to clarify the role of the dysfunctional C3 in this autoimmune condition.

Patient and methods

CASE REPORT

The patient received plasma from her husband, who had plasmapheresis before each session. Both the patient and her husband were 0 Rhesus positive. The husband had not received blood transfusions or any other blood products which reduced the possibility that he was immunised against leukocyte antigens. In vitro analyses confirmed that the plasma contained no such antibodies (agglutination and cytotoxic assays). The plasma was always given through a leukocyte depletion filter to avoid adverse reactions and immunisations against leukocyte antigens. The study was approved by the Ethical Committee of the Medical Faculty, Uppsala University. The patient gave her informed consent to participate in the study.

The plasma treatment was performed during five periods (table).

Session 1

The first dose was taken well but six hours after the second dose the patient developed fever, nausea and diarrhoea which continued until the next day. There was also transient mild haematuria (+) and proteinuria (1+). A Coombs test showed no erythrocyte-bound IgG or C3d before or after the transfusions.

SESSIONS 2–5

A minute inflammatory reaction was observed after the second, third and fourth treatment period in that the fingers were more swollen and the arthralgia was worse.

The treatment during the eight month period resulted in no obvious clinical improvement of the propositus clinical condition except that the patient’s Raynaud’s phenomena seemed to disappear. The disease activity assessed by the SLE Disease Activity Index (SLEDAI)6 remained at the same level before and after the treatment period (score 6). However, the patient has subjectively improved and the fatigue has vanished. Before the treatment the propositus was unable to work for five years but she has now returned to her previous occupation as a physiotherapist.
Nilsson, Nilsson, Karlsson-Parra, Sjölin-Fosberg, Hallgren

**Complement profiles of the propositus during the five plasma infusion treatment**

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<th>CPW (%) (80–120)</th>
<th>APW (%) (50–150)</th>
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<th>C4 g/L (0.09–0.29)</th>
<th>FB g/L (0.16–0.44)</th>
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**Figure 1** Monitoring of C3 (●) and APW (○) [A]; C3a (●) and TNF-α (○) [B]; C3-containing ICs (●) and IgG-containing ICs (○) [C] throughout the five plasma treatment sessions.

**BLOOD SAMPLING**
Serum and EDTA-plasma were separated from the blood samples, frozen and stored at −70°C within two hours from venipuncture.

**LABORATORY TESTS**
Quantitative immunochemical analysis of C3, C4, factor B and C3d was performed on a Beckman Array Nephelometer according to the manufacturer’s recommendations. C3d was assessed in the supernatant after PEG precipitation. C3a was analysed as described in Nilsson Ekdahl et al. TNF-α and IL-6 were assessed by ELISA from Medgenix, Belgium. IgG and C3-containing immune complexes were assayed in PEG-precipitated samples, described previously. Haemolytic assays of the complement function were performed according to Nilsson and Nilsson.

**Results**

**HAEMOLYTIC FUNCTIONS AND LEVELS OF C3, C4 AND FB DURING PLASMA INFUSIONS**
Figure 1A and the table record the levels of APW and CPW function and the levels of C3, C4 and FB during the plasma treatment. APW was fully restored (normal range 50–150%) after the second, third and fourth infusion periods while only a partial reconstitution occurred after the first and fifth treatment. Elevated APW function lasted approximately five to six days as estimated from extrapolation of APW function after the second, third and fourth infusion periods.

**GENERATION OF C3 ACTIVATION FRAGMENTS DURING PLASMA INFUSIONS**
The activation fragments C3a (fig 1B) and C3d (table) were assessed during the plasma infusions. The C3a levels increased from almost normal levels (<200 ng/ml) to 950 ng/ml after the first session. After the next four treatments the increases gradually declined to moderate...
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Figure 2  Tissue deposition of C3 fragments during the second session of plasma infusions. No significant deposits of C3 was found before the session was started (fig 2A). Two days after the second plasma treatment when 4 x 500 ml of plasma were infused, biopsies taken close to the previous biopsies, showed significant granular deposits of C3 fragments at the same location as previously found IgG and IgM deposits (fig 2B).

development of C3 fragments in the skin after plasma infusions

Before the second session of plasma infusions, skin biopsies showed deposition of IgG and IgM at the dermis-epidermis junction. No significant deposits of C3 was found (fig 2A). After the second plasma treatment, biopsies taken close to the previous biopsies, showed significant granular deposits of C3 fragments at the same location as the immunoglobulins (fig 2B).

generation of TNF-α and IL-6 during plasma treatment

During each session of plasma infusion the levels of TNF-α increased rapidly and returned back to normal levels of approximately 20 pg/ml after each period (fig 1B). The patients IL-6 levels were consistently increased already before plasma infusions (56–113 ng/ml). The concentrations increased after session II-IV with peak levels ranging from 93 to 234 ng/ml (not shown).

serum IC-C3 and IC-IgG levels

Initially, the serum levels of IgG- and C3-
containing ICs were exceedingly high with peak levels reaching 9.0 µg/ml and 0.7 µg/ml, respectively (normal values <1.2 µg/ml [IgG]; <94 ng/ml [C3]). During the plasma treatment the levels gradually decreased and were half the original levels after the last plasma infusion (fig 1C). During the year preceding the plasma treatment the IC levels were assessed four times ranging between 8.9–9.5 µg/ml and 0.6–0.8 µg/ml, respectively.

HAEMATOLOGICAL AND INFLAMMATORY PARAMETERS

No consistent changes in the counts of mononuclear or polymuclear leucocytes, eosinophilic granulocytes or platelets were observed. The haemoglobin concentration did not change (1 and 5) or decreased, due to dilution, to a nadir of 88% of the original level (2–4). The sedimentation rate was not increased and C reactive protein was marginally elevated during the five sessions.

Discussion

Reconstitution with complement components of patients with hereditary complement deficiencies combined with SLE-like syndromes may prove therapeutically beneficial, since complement is crucial for the handling and elimination of IC. A precedent to plasma treatment was observed in a case of therapy-resistant SLE with a homozygous C2 deficiency. Full clinical remission was seen over a period of 45 months involving repeated cycles of fresh plasma therapy. The purpose of the present study was to find evidence that reconstitution by plasma could give rise to a functional alternative pathway in the propositus. During three of these periods full reconstitution was achieved, that is, >50% function by the APW. Complement activation by immune complexes, as detected by increased C3a levels, was observed during each treatment period. The levels of C3a did not correlate to the amount of infused plasma. The levels were most elevated during the first treatment period and thereafter the peak value declined during the two subsequent treatments which suggest that the C3a peak levels reflect the total immune complex load. As an indication that tissue bound immune complexes activated complement, deposition of C3 was observed in the skin after the second plasma treatment. This observation suggests that a functional alternative pathway is necessary to deposit C3 into the IgG and IgM deposits at the dermal-epidermal junction.

During each plasma infusion period TNF-α and IL-6 increased as indicators of cellular activation. Ingestion of immune complexes by the fixed macrophage system is likely to contribute to such an activation since the levels of plasma ICs gradually decreased to half of the original levels during the whole treatment period.

The clinical efficacy of the treatment when formally assessed was not obvious. However, the fever, which was the most disabling symptom, gradually disappeared and after plasma therapy our patient has experienced improved well being and function, including working capacity and the ability to carry out activities of daily living. These therapeutic effects might be the result of a decreased immune complex load.

However, the possibility exists that plasma infusions could actually worsen tissue damage. Our patient responded with fever, nausea, vomiting, diarrhea and a transient haematuria and proteinuria after the first plasma infusion session during which the patient received only 500 ml of plasma. These findings indicate that plasma treatment might be potentially hazardous and suggest that such treatment in patients with complement deficiencies should be carried out with initially very low doses followed by gradually increased amounts.

The excellent technical assistance by Mariette Siuunnesskog and Eva Alriksson is gratefully acknowledged. This study was supported by the Swedish Medical Research Council, grant 5647.

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