Complement has two main roles as part of the human innate immune system. Historically, the first of these to be defined was that of defence against infection with pyogenic bacteria. However, it is now evident that complement proteins, particularly those of the classical pathway, play a role in the processing of immune complexes (ICs) and in protecting the body against the development of diseases such as systemic lupus erythematosus (SLE), which are thought to be mediated by ICs. The study of individuals with inherited deficiencies of individual complement proteins has provided many insights into the functions of these proteins in vivo. The most convincing evidence that complement is important in protection against IC disease follows from the observation that the prevalence of certain diseases in which ICs are a characteristic feature, particularly SLE, is greatly increased in individuals who are genetically deficient in classical pathway complement proteins [1].

In this essay, I plan to discuss the results of the work I have performed during the last 5 yr in the Rheumatology Unit at the Royal Postgraduate Medical School (RPMS). During this time we have explored specifically the interactions between ICs and the proteins of the complement system and their cellular receptors in the pathogenesis of SLE. After a brief discussion of the background to the work, I describe some of the key in vivo and in vitro experiments and clinical studies we have performed, which have advanced our understanding in this area, and at the end of the dissertation I attempt to address some of the implications of our observations for the management of patients with SLE.

Immune Complex Processing

What was the state of knowledge of the role of complement and complement receptors in the processing of ICs in the late 1980s, when I first became involved in research in this area? It was well known that complement reacted with ICs to inhibit immune precipitation, solubilize immune aggregates and promote IC binding to erythrocyte complement receptor type 1 (CR1). These reactions were thought to prevent local accumulation of ICs in tissues outside the mononuclear phagocytic system by producing soluble complexes which are removed by simple diffusion away from the site of formation and by binding to erythrocyte CR1. This receptor decreases the phlogistic potential of ICs by: (i) acting as a cofactor to the alternative pathway control protein, Factor I, which facilitates the further breakdown of the most important complement protein C3; and (ii) transporting ICs through the circulation to the fixed mononuclear phagocytic system. Tissue macrophages in the liver and spleen bear both immunoglobulin Fc- and complement receptors type 3 and 4 (CR3 and CR4), and opsonized complexes can interact with both these groups of receptors. It had long been mooted that there may be a primary or acquired defect in mononuclear phagocytic function in SLE which predisposes to the development of disease by impairment of complex clearance. This idea that abnormal function of the reticuloendothelial system might result in failure of IC processing stemmed from early experimental work in animals by Biozzi and colleagues using colloidal carbon particles [2], and by Haakenstadt and Mannik [3], who demonstrated that IC injection into rabbits resulted in saturable hepatic uptake, followed by spillover into other organs. Whether or not this so-called 'reticuloendothelial saturation' occurs in humans and is a contributory factor in the development of disease is not clear. Much experimental effort has been devoted to addressing the question of whether there is indeed a fundamental abnormality of mononuclear phagocytic system function in SLE related primarily to IC clearance mediated by Fc receptors [4], or whether the primary problem is one of defective IC delivery to the mononuclear phagocytic system secondary to hypocomplementaemia and/or low levels of erythrocyte CR1, as suggested by Peter Lachmann in 1987 [5]. The experimental work which I describe in the first part of this essay attempts specifically to resolve this question.

A number of different model systems had been employed to address this problem previously. Early studies used erythrocytes coated with IgG or IgM, and more recent studies have employed either aggregated immunoglobulin or soluble ICs [4, 6-8]. Erythrocytes coated with IgG are cleared in the spleen (Fc receptor dependent) [4, 9], while IgM-coated cells show transient retention in the liver mediated by reversible binding to complement receptors [10]. The clearance of IgG-coated erythrocytes has been specifically studied in SLE patients. Frank and colleagues [11, 12] demonstrated a correlation between clearance rate, disease activity and levels of circulating ICs in patients with SLE, but a number of other similar studies have failed to show any such direct correlations [13-15]. Mark
Walport showed in 1985 that splenic blood flow is an important factor affecting the clearance of IgG-coated red cells [16], and this may, of course, influence disease-associated clearance of these cells.

More recently, Lobatto and colleagues [6] assessed mononuclear phagocyte function in different diseases, using radiolabelled soluble aggregates of IgG. These aggregates were predominantly cleared from the circulation in the liver and spleen. Significant differences were seen between normal subjects and patients with SLE. In particular, the liver/spleen uptake ratios were higher in the patients, due to reduced splenic uptake of the aggregates. Halma and colleagues [17] analysed clearance of aggregated IgG in 22 patients with SLE and 12 normal volunteers, demonstrating reduced binding of aggregates to red cell CR1 in the patient group, with a faster initial elimination rate. In this study, the major factor influencing the aggregate clearance rate was the serum IgG concentration.

Soluble IC clearance had been studied by Schifferli and co-workers [8] in our unit using 125I-labelled tetanus toxoid/anti-tetanus toxoid complexes [8]. Either native complexes, or complexes pre-opsonized in vitro with autologous serum, were injected into normal volunteers, and into 15 patients with IC disease or hypocomplementaemia. Immune complexes bound to erythrocyte CR1 receptors in a complement-dependent manner, and CR1 number correlated with the level of uptake. In subjects with low CR1 numbers and hypocomplementaemia, there was a very rapid initial disappearance of IC, which was attributed at that time to possible deposition of complexes outside the reticuloendothelial system, at other sites where they might be potentially harmful, although in this early work no direct imaging of the sites of IC clearance was performed. Two critical questions remained unanswered at this time: (1) to what extent are the results of these clearance studies using pre-formed ICs applicable to the situation which exists physiologically, in which the complexes are actually formed in vivo in the presence of complement proteins, which have profound effects on their physicochemical properties (discussed in detail in a recent review written in collaboration with Professor Schifferli [18]); and (2) where are soluble ICs really cleared in humans?

One of the criticisms of all the studies of IC processing described above is that they were all performed using large ICs prepared in vitro, in the absence of complement, and may not therefore be physiological. There is conflicting evidence regarding the binding of ICs formed in the presence of complement to erythrocyte CR1. Varga and colleagues [19] demonstrated that bovine serum albumin (BSA)-anti-BSA complexes formed in the presence of serum failed to bind to erythrocytes. However, others have shown that the successive infusion of human dsDNA antibodies and dsDNA into monkeys and rabbits leads to the rapid formation of ICs capable of binding to red cell CR1 [20]. In human IC disease, it is inevitable that complexes will be formed in vivo, either in the circulation, or at a site of inflammation and autoantigen presentation, in the presence of complement, and in the first project upon which I embarked when I started as an Arthritis and Rheumatism Council junior fellow in Mark Walport’s laboratory at the Hammersmith, I attempted to address, for the first time, the fate of ICs formed in vivo in man.

How did we approach this problem? We needed to find a clinical situation in which it was likely that complexes would be formed in the circulation, ideally as a consequence of planned therapeutic intervention of some sort. The classical models of in vivo IC formation in animals involved the induction of serum sickness and IC-mediated nephritis [21]. The administration of an exogenous antigen, in the form of a drug, to a pre-immune individual could clearly produce similar effects in man, as we demonstrated in a patient who had been pre-sensitized, and then received i.v. streptokinase therapy [22]. A model was therefore required in which ‘safe’ complexes were formed, ideally in a situation in which their formation and clearance could be monitored in some way. It was Dr Epenetos, from the Department of Clinical Oncology here at the RPMS, who provided us with the model we needed. He had recently started treating his ovarian cancer patients with a new form of radioimmunotherapy. Treatment comprised the following: under general anaesthetic, mouse IgG1 monoclonal anti-tumour antibody (anti-human milk fat globule 1, HMFG1), at a dose of 10–12 mg, labelled with 131I, was injected i.p. via a rigid catheter (of the type designed for peritoneal dialysis) under direct laparoscopic vision on day 0. Two doses (15–18 mg) of 125I-labelled antibody to mouse...
immunoglobulin were administered over 15–20 min by i.v. infusion into a peripheral vein, 24 h after the first (anti-tumour) antibody (Fig. 1) given in order to accelerate the clearance of the first antibody and reduce potential radiotoxicity from anti-HMFG1 antibody that did not bind to the tumour. This model facilitated the analysis of the formation and clearance of ICs formed in the circulation from the two antibody species.

Three patients (patients 1, 2 and 3) aged 43, 44 and 42 yr were studied. All three were systemically entirely well at the onset of therapy, but had advanced ovarian carcinoma with evidence of peritoneal spread. Treatment was given as part of a trial of radioimmunotherapy in ovarian cancer approved by the Hammersmith Hospital ethics committee. Sequential samples of venous blood were obtained from all three patients before therapy and at 5 min intervals for 1 h after starting infusion of the human anti-mouse antibody. Additional samples were obtained 12 and 24 h later, and at 1–2 week follow-up visits. We monitored the patient externally with a gamma counter over the heart and abdomen, in order to obtain an idea of their main site of localization.

The use of this model system, in which ICs were formed in vivo between an antigen and an antibody labelled with two different radioisotopes, one at a high specific activity, facilitated the detailed characterization both of the kinetics of complex formation and disposal, and the way in which these complexes interacted with CR1 on erythrocytes. An acquired reduction in erythrocyte CR1 numbers has been described by a number of different groups in patients with SLE [23, 24, 25], and it had been postulated (i) that such a reduction may occur as a consequence of IC ligation by CR1, transport to the liver and spleen, and proteolytic removal of both the complex and part or all of the receptor as a consequence of this interaction between the IC-E-CR1 complex and the cells of the fixed macrophage system, and (ii) that this acquired reduction in receptor number may be one of the factors contributing to abnormal IC processing in patients with this condition [5]. In the radioimmunotherapy model, in which relatively large quantities of ICs were formed in vivo, two of the key questions which I set out to address were whether erythrocyte CR1 are indeed implicated in the clearance of complexes, and whether a fall in receptor numbers could be demonstrated consequent upon IC formation and clearance. Systemic complement activation, and deposition of the complement fragments C4d, C3dg and iC3b on the erythrocyte surface, have also been demonstrated in patients with SLE by ourselves and others [26–28], and in the radioimmunotherapy model we sought evidence for similar changes, occurring more acutely, following IC formation.

A full account of this work may be found in our paper published in 1990 in the *Journal of Immunology* [29]. Our results confirmed that systemic complement activation, and deposition of C4 and C3 fragments on erythrocytes, did indeed occur (Figs 2 and 3). It was also demonstrated by sucrose gradient analysis that the complexes formed comprised both antibody species (Fig. 4), and that the clearance of the first antibody was greatly accelerated as a consequence of IC formation.

---

**Fig. 2.**—Fluid-phase complement activation after IC formation in vivo.

**Fig. 3.**—Changes in erythrocyte CR1, C3dg and C4d following in vivo formation of ICs in the radioimmunotherapy model.
From studies on the patients' red cells, it was clear that a fraction of the complexes bound to erythrocyte CR1 (Fig. 5). We also showed that these in vivo-formed complexes were cleared mainly in the liver, although a relatively crude method of external monitoring was employed. Perhaps the most exciting finding in this study was the observation that in all the subjects studied, a fall of up to 30% of the initial value in CR1 could be demonstrated following infusion of the second antibody, and IC formation and clearance (Fig. 3). In parallel in vitro studies, employing the same radio-labelled antigen/antibody system, we were able to demonstrate analogous complement deposition on red cells, and fluid phase complement activation, but no fall in erythrocyte CR1. This would strongly suggest that it is indeed the process of IC removal from the erythrocyte by cells of the fixed macrophage system that is responsible for CR1 loss, and that this is not merely a consequence of E-CR1 ligation by C3-opsonized complexes. In the in vivo studies, only a small number of complexes could be demonstrated on a red cell at any one time (1–3 complexes per cell) and it is, at first sight, difficult to imagine how the process of IC clearance could result in the loss of a significant number of CR1, as we demonstrated. It should be remembered, however, that the processes of IC formation and clearance in vivo are dynamic ones, and red cells binding complement and IC on their surface are able to interact sequentially with cells of the fixed macrophage system, with stripping, and subsequent re-binding of other complexes. It is also important to note that the distribution of CR1 is not homogeneous on the erythrocyte surface [31]. It is clear from electron microscopy studies that the CR1 are clustered in small groups of between 3 and 12 molecules. All red cells exhibit this clustering, though to a variable degree: the cells bearing the highest CR1 number are generally those having the largest clusters, and are probably the most efficient at binding ICs. Cosio and colleagues [32] analysed the binding of ICs to red cells using an immunofluorescence technique. It was observed that only some erythrocytes are capable of binding ICs, and as in the radioimmunotherapy model system only relatively few ICs bind per erythrocyte, suggesting that the binding was restricted to defined areas on the cell surface. It is therefore likely that the ligation and subsequent stripping of a large C3b-opsonized antibody-antibody complex, as in this model, would result in the loss of multiple CR1.

Further evidence for the loss of CR1 from red cells by proteolytic mechanisms comes from the work of Barbosa and colleagues, who suggested that CR1 are degraded in vivo to leave only 'stumps' consisting of transmembrane and intracellular domains of the molecule, to which he developed a specific monoclonal antibody [33].

![Sucrose density-gradient profiles from patient 3, following human anti-mouse antibody injection.](https://academic.oup.com/rheumatology/article-abstract/35/1/5/1782225/1782225)

**Fig. 4.**—Sucrose density-gradient profiles from patient 3, following human anti-mouse antibody injection.

![Clearance of 131I-labelled antigen following injection of human anti-mouse antibody; TCA and polyethylene glycol (PEG) precipitation. Erythrocyte antigen binding is shown on an expanded scale on the insert graph.](https://academic.oup.com/rheumatology/article-abstract/35/1/5/1782225/1782225)

**Fig. 5.**—Clearance of 131I-labelled antigen following injection of human anti-mouse antibody; TCA and polyethylene glycol (PEG) precipitation. Erythrocyte antigen binding is shown on an expanded scale on the insert graph.
Low CR1 numbers have been described on erythrocytes of patients with a variety of diseases other than SLE, including paroxysmal nocturnal haemoglobinuria, autoimmune haemolytic anaemias, AIDS and lepromatous leprosy. The mechanisms of CR1 reduction in AIDS patients have recently been studied in detail. Patients with HIV infection have a progressive loss of CR1 on red cells when the infection progresses to AIDS [34-36], and genetic studies have confirmed that the defect is acquired [37]. These patients do not have a loss of glycosylphosphatidylinositol (GPI)-anchored proteins, and the pattern of CR1 fragments on erythrocytes is different from that on normal ageing erythrocytes, indicating that in AIDS the reduced number of CR1 is due to enhanced proteolytic cleavage [38]. All these observations support the hypothesis that the reduction of erythrocyte CR1 in 'IC' diseases is indeed related to proteolytic loss of the receptor as a consequence of IC processing.

STUDIES IN PATIENTS WITH SLE

Our radioimmunotherapy study strongly suggested that the main site of IC clearance in man was the liver. In the next phase of our work, we developed a model using radiolabelled pre-formed ICs, of high specific activity, with a view to determining definitively the sites of soluble complex clearance in man. We also planned to use this model to examine further the clearance of ICs in patients with SLE, and to analyse the ways in which the kinetics and sites of complex clearance were influenced by low serum complement levels, and low levels of erythrocyte CR1 in the disease. These studies were the first to be performed in man in which the sites of clearance of soluble ICs were directly visualized using an imaging technique, and were reported by our group in the Journal of Clinical Investigation [40]. The complexes used comprised hepatitis B antigen (HBsAg) and a polyclonal anti-hepatitis B reagent. These model complexes comprise components specifically prepared and licensed for use in humans (the antigen is the material used to prepare the vaccine, and the antibody is an approved preparation from the Swiss Red Cross which is normally used for passive immunization). Radiolabelling with $^{125}$I (an isotope with a short half-life, which has an emission spectrum ideal for imaging studies) was performed using the N-bromosuccinimide method [39]. Twenty-six subjects were studied—eight males and 18 females. Twelve normal volunteers were recruited from the laboratory staff, aged from 23 to 59 yr, six female and six male. The patients were 10 subjects with SLE, aged from 21 to 56. Nine were female and one male. All fulfilled the revised ACR criteria for this disease. A 'butterfly' (19G) i.v. cannula was inserted into the subject's left antecubital fossa for injection of radiolabelled complexes, and a similar line in the right arm for blood sampling. Five millilitres of IC were injected as a bolus over 20 s at time 0, with the patient positioned under a gamma camera (IGE 400T on line to an MDS A2 computer). Dynamic imaging was performed (serial 20 s frames) for 50 min, followed by 5 min posterior and anterior static images at 1, 4 and 24 h. Four subjects underwent dynamic scanning for 2 h, and whole-body scanning was also performed in two cases, using a Starcam (IGE) gamma camera.

During scanning, sequential blood samples were taken for measurement of whole-blood $^{125}$I, trichloroacetic acid (TCA) precipitation of protein-bound activity, estimation of erythrocyte-bound $^{125}$I and complement assays. Immune complexes were characterized by co-precipitation with staphylococcal protein A and sucrose density-gradient centrifugation. Erythrocyte CR1 numbers were measured using a radioligand binding assay with an anti-CR1 monoclonal antibody [41]. The quantification of IC uptake in the liver and spleen was performed from radioactivity count rates in specific 'regions of interest' (ROI) drawn around each organ on anterior and posterior images. Both SLE patients and controls exhibited uptake of labelled ICs in the liver and spleen, with rapid clearance from the blood (Fig. 6). Scanning over the lungs, kidneys and other organs revealed no specific uptake above blood pool level in either group (Fig. 7). The initial rate of IC clearance from the blood was significantly faster in the SLE group (median $t_{1/2} = 2.15$ min, range 1.3–6.6 min) than in the normal controls (median $t_{1/2} = 5.15$ min, range 3.6–14).
The median time at which 90% of maximum hepatic uptake was reached in the SLE group was 9.0 min (range 4.3–18 min), compared with 16.0 min (13–23 min) in the normal subjects ($U = 8.0$, $P < 0.002$) (Fig. 8B). At 10 min, between 27.3 and 67.5% (median 40.7%) of injected complexes could be detected in the liver in the normal subjects, compared with 43.0–79.6% of injected IC (median 56.3%) in the patient group ($U = 27$, $P < 0.05$).

In both normal subjects and in the SLE group, a fall in hepatic activity was observed between 30 min and 2 h after injection. This was more marked in the patients, as demonstrated by comparison of the ratio of hepatic counts measured at 40 and 60 min in the two groups—median ratio 1.24 (range 0.88–1.44) in the studies performed in patients with SLE, compared with 0.75 (0.48–0.95) in the controls ($U = 2$, $P < 0.02$). These data are shown in Fig. 9.

The measurement of whole-blood, protein-bound and red cell-bound activity in sequential samples following injection of labelled IC indicated that there was significant release of protein-bound radioactivity from the liver in the patient group occurring after 30–40 min. In Fig. 10, in which the patterns of IC handling in a normal subject and a typical SLE patient are compared, there is a clear rise in the whole-blood and TCA-precipitable activity between 25 and 60 min. The TCA precipitation data indicated that a significantly greater proportion of the activity detected in the blood at 1 h was protein bound in the SLE patients (median 86.7%, range 77.0–96.7) than in the normal controls (median 69.7%, range 52.0–79.1) ($U = 2.0$, $P < 0.02$). Precipitation of this material using Sepharose-staphylococcal protein A showed that the $^{125}$IHBsAg remained complexed with IgG. The size of this material was estimated by sucrose density-gradient centrifugation, which showed it to be composed primarily of material of between 35 and 50S—intermediate between antigen and the injected ICs (Fig. 11a and c).
There was significantly reduced uptake of ICs by the spleen during the first hour amongst the patients (median 9.03% of injected ICs, range 4.05-23.7%) compared with the normal controls (median 23.9%, range 17.9-30.7%) (U = 4, P < 0.02). The ability of the spleen to retain ICs following initial uptake into the organ was also abnormal in the SLE patients. The activity remaining in the spleen at 24 h was expressed as a percentage of the maximum organ uptake measured during the first hour after IC injection. Amongst the SLE patients, median uptake was 39% maximum (range 24-52%) compared with normal subjects—median 65.5% (range 58-73%) (U = 0, P < 0.002) (Fig. 12a, uptake at 1 h as a percentage of the maximum counts in the organ).

The major differences in the processing of ICs between the normal subjects and patients with SLE can be summarized as follows: (i) initial clearance of ICs was more rapid in patients than in controls, as complexes localized rapidly in the liver; (ii) IC release from the liver occurred subsequently in patients; (iii) there was significantly reduced splenic complex uptake in the patient group. The various different factors which might explain these differences were therefore examined. The median erythrocyte CR1 number measured in the normal control group was 950 (range 467-1218). The median value for the SLE patients was significantly lower at 482, range 78-969 (U = 19, P < 0.02). In all subjects, there was a very close linear correlation between the maximum binding in vivo of IC to erythrocytes and the CR1 number (r = 0.96). CR1 numbers on erythrocytes were also correlated with the rate of clearance of ICs by the liver (Fig. 13).

In the patients with SLE, there was a close correlation between the levels of both C4 and C3 in the plasma and the t₁/₂ of IC clearance—C4: r = 0.997; C3: r = 0.811. The correlation for C4 is illustrated in Fig. 14. These data suggest that hypocomplementaemia might play an important role in determining the abnormal IC kinetics amongst the patients with SLE. We considered the possibility that this link was related to the IC size, which we measured by sucrose density-gradient analysis. Figure 12 shows typical data. A shift in peak complex size was seen in the 3 and 5 min samples obtained from a normal subject, which was not observed in the samples from an SLE patient.

These imaging studies provided direct evidence that a reduction in plasma complement levels and erythrocyte CR1 profoundly affects in vivo processing of ICs. Hypocomplementaemia was associated with more rapid uptake of complexes into the liver, with subsequent release into the circulation of IC of intermediate size. As we have previously discussed, it had been noted in earlier studies using 125I-labelled tetanus toxoid/anti-tetanus toxoid complexes [8] that there was more rapid initial clearance of ICs from the circulation.
in patients with SLE, or C1q deficiency. This was attributed to 'trapping' of complexes outside the reticuloendothelial system, but in these earlier studies direct imaging was not available. In our imaging experiments, we saw no evidence of 'trapping' outside the liver and spleen. The markedly increased rapidity of initial clearance from the circulation was due to more rapid hepatic uptake. There was then release of ICs from the liver following this initial uptake phase. We do not know the true significance of this observation, but clearly material of this sort could have phlogistic potential in itself, or serve to modulate autoantibody production. It is tempting to speculate that ligation of both Fc and complement receptors is required for efficient processing of complexes, a hypothesis that we are actively investigating.

![Graph](https://academic.oup.com/rheumatology/article-abstract/35/1/5/1782225)

**Fig. 11.** (a) SDG profiles generated by labelled Ag alone and by the IC preparation before injection; (b) analysis of plasma samples from a patient and normal at 0, 3 and 5 min; (c) profiles at 0, 3 and 50 min, showing material of intermediate size at 50 min.
STUDIES IN A C2-DEFICIENT PATIENT

From the above studies, it appeared that complement levels affected both the sites and kinetics of IC clearance in SLE. Our next step was to use the same model system to study IC processing in a patient with homozygous C2 deficiency and SLE, exploring the effects on IC processing of the repletion of normal complement activity with fresh frozen plasma (FFP).

This work has recently been reported in the Journal of Immunology [43]. The patient described in the study has been reported previously and has homozygous C2 deficiency [42]. She is under the care of Dr Kristian Erlendsson, University Hospital, Reykjavik, Iceland. Dr Erlendsson kindly accompanied her on her visit to the Hammersmith Hospital for the performance of the scans, and also supervised her therapy with FFP. She was 53 yr old at the time the study was performed. Her original presentation in 1982 to the Department of Internal Medicine and Clinical Immunology, University Hospital, Reykjavik, was with a butterfly facial rash, severe Raynaud's, a symmetrical polyarthritis and alopecia. The patient's symptoms initially improved with hydroxychloroquine and prednisolone, but in 1988 she relapsed with a severe photosensitive rash and digital gangrene. At this stage, therapy with regular infusions of FFP (3 units administered daily for 4 days, every 6–8 weeks) was instituted. Each treatment resulted in marked improvement in her general well-being for up to 2 months, with rapid resolution on each occasion of her rash and digital vasculitic lesions.
Initiation of this treatment regime facilitated cessation of all drug therapy. At the time she was studied at the Hammersmith Hospital, she was well, with no rash or other specific manifestations of her disease. She was receiving FFP at ~8 week intervals, and was transferred to our care for her first IC study 4 days before she was due to receive a further series of infusions. The protocol for the study was as shown in Fig. 15. Control subjects were healthy volunteers from the laboratory, one male and one female, aged 34 and 32 yr, respectively.

In the IC scanning study performed before treatment with FFP therapy, there was rapid uptake of labelled complexes into the liver [t90% (time for 90% uptake) = 13.6 min], corresponding to their rapid clearance from the circulation—t1/2 = 6.8 min (Fig. 16). No uptake in the spleen was detected (Fig. 17). Between 30 and 60 min, there was release of the tracer from the liver. This had been demonstrated in the previous studies performed in lupus patients to be due to release of labelled ICs of intermediate size back into the circulation. We did not observe this phenomenon in a control subject studied with the same labelled complexes on the same day. Values in the control subject for t1/2 and t90% were 12.5 and 26 min, respectively (Fig. 16B). A maximum of 21% of injected IC localized in the spleen in the control subject.

The second IC scanning study was performed on day 8, immediately after the completion of the last infusion of FFP. The observed pattern of complex uptake and processing was markedly different in this second study, resembling that observed in the control subject. t1/2 of IC clearance was 9.8 min and t90% was 27 min (Fig. 18). Twenty per cent of injected complexes localized to the spleen (Fig. 19), and there was no release between 30 and 60 min.

We addressed the questions of whether there was a generalized defect in the function of the mononuclear phagocytic system, or a reduction in splenic blood flow in the patient (as has been described in SLE previously), which might explain the impaired splenic uptake of ICs. This was done by performing 99mTc-colloid scans on days 2 and 9. The spleen/liver uptake ratio was 0.131 in the pre-treatment study and 0.132 in the second study. Both values are within the normal range for the tracer used. The two scans obtained were

![Fig. 16. Kinetics of IC disposal in the C2-deficient patient before treatment (A) and in a normal subject (B).](https://academic.oup.com/rheumatology/article-abstract/35/1/5/1782225)

![Fig. 17. Immune complex scan in the C2-deficient patient, before therapy (left: anterior; right: posterior).](https://academic.oup.com/rheumatology/article-abstract/35/1/5/1782225)
almost identical in appearance and the computer-generated liver:spleen profile curves were very similar (not shown).

Immune complex size was estimated prior to their injection and in plasma obtained from the patient at 3 and 5 min. In the C2-deficient patient, there was no obvious change in size subsequent to injection (Fig. 20A), while in the normal control, the majority of the labelled complexes detected in the circulation at 5 min were smaller than those injected—demonstrated by a shift in the gradient peak in Fig. 22. Following FFP therapy, and the restoration of serum complement, a similar shift could be demonstrated between 0 and 5 min in the patient (Fig. 20C).

The binding of ICs to erythrocytes was measured in serial blood samples after IC injection. The clearance of protein-bound tracer from the circulation, in the studies performed before and after FFP therapy in the patient, is shown in Fig. 21. Less than 2% of the injected complexes were bound to erythrocyte CR1 at 2 min in the pre-treatment study, while 72% of protein-bound activity was measured on erythrocytes at 2 min in the study performed after normalization of serum complement levels.

There was markedly reduced uptake and retention of complexes in the spleen in the SLE patients, and the pre- and post-treatment studies in the Icelandic C2-deficient patient demonstrated for the first time in humans that the uptake of ICs in the spleen is a complement-dependent phenomenon. Similar observations were made in the 1970s by Pepys and colleagues [44–46], and complement has been demonstrated in a number of model systems to be important for the localization of certain antigens to follicular dendritic cells, and within splenic lymphoid follicles. In guinea-pigs, the spleen has also been shown to be of major importance for the clearance of complement-opsonized bacteria [47]. Our observations relating to the complement-dependent nature of splenic IC processing in humans have both immunopathological and clinical implications. Failure to localize ICs and their antigens in the spleen in SLE may have a bearing on the production of antibodies in this condition, either to self or exogenous antigens. Efficient and safe processing of certain bacterial or viral antigens, or autoantigens derived, for example, from apoptotic leucocytes, may depend on their uptake in the spleen, localization to the appropriate antigen-presenting cells and the rapid production of antibody. It is tempting to speculate that persistence of either exogenous or autoantigens as a result of impaired clearance in the reticuloendothelial system might result in the presentation of antigen to 'non-professional' antigen-presenting cells elsewhere in the immune system, resulting in an abnormal cellular or humoral immune response. Alternatively, the 'planting' of a persistent antigen, for example in the kidney, might then result in the in situ formation of potentially harmful ICs, as has been demonstrated in various experimental models of glomerulonephritis [48]. I speculated above that there may be a primary abnormality in Fc-mediated reticuloendothelial function in SLE, as has been suggested by Kimberly and his co-workers, who performed many of the original studies of antibody-coated erythrocyte clearance. The observations that the handling of sulphur-colloid (pre-FFP therapy) was completely normal in our Icelandic C2-deficient patient, and that the abnormalities of both
the kinetics and sites of IC clearance seen pre-treatment were totally correctable by normalizing her classical pathway complement function with FFP, would seem to mitigate against there being a primary abnormality of reticuloendothelial function, at least in this patient.

**CLINICAL IMPLICATIONS**

**SLE and infection**

What are the clinical implications of the observations made in our studies of in vivo IC processing in SLE and C2 deficiency? Firstly, we might consider to what extent our observations may be extrapolated to the clearance of ICs comprising antibody and bacteria. Infection is a major cause of morbidity in patients with SLE, and in two recent large studies of mortality in SLE, sepsis was implicated as a major cause of death [49, 50]. More specifically, there are many reports in the literature of fatal overwhelming infections with both *Streptococcus pneumoniae* and *Neisseria meningitidis*, in patients with SLE [51]. While steroids and immunosuppressive therapy may be contributory factors in these cases, it is possible that functional hyposplenism, related to defective splenic clearance of bacterial ICs, may also also of major importance. It is particularly interesting in this context that SLE patients are susceptible to a very similar spectrum of infections by bacterial pathogens, as patients who have had a surgical splenectomy, notably pneumococci. Pathogenic *S. pneumoniae* are encapsulated, and are resistant to direct complement-mediated opsonization and phagocytosis [52]. The pneumococcal cell wall can bind opsonically active C3b and iC3b, but there is evidence that the capsule masks polymorph access to the bound complement components. It is tempting to speculate that the processing of these particulate pathogens in the spleen is critical both for their removal from the circulation in the event of bacteraemia, and for the development of an appropriate antibody response. Hypocomplementaemia will result in defective opsonization, with both a reduction in the local phagocytosis and delivery to the main site of processing of particulate antigens, the spleen. Genetic deficiency of plasma complement proteins (of the classical, alternative and terminal pathways [1, 53, 54]), or of leucocyte complement receptors CR3 and CR4 (as in 'leucocyte adhesion deficiency',...
LAD [55] are strongly associated with the development of severe infections, and we suggest that the acquired hypocomplementaemia of SLE may also put patients at risk of serious infection. Do we have any direct evidence for this suggestion? We have recently identified a strong association between hypocomplementaemia in SLE and the presence of antibodies to the first complement component, C1q [56]. The latter are found in around 25–30% of patients with SLE [57], and we are currently studying both the diagnostic utility and potential pathogenetic role of this autoantibody in SLE. Our preliminary findings were presented at the recent British Society for Rheumatology Annual Meeting [58]. One alarming, though perhaps not entirely surprising, association which we identified with anti-C1q antibodies and low complement levels was with sudden septic death in patients with SLE. Of the four patients with SLE from our unit who have died over the last 5 yr from infection, one had homozygous deficiency of C1q (reported recently in the Quarterly Journal of Medicine [59]), and the other three had persistently low complement levels and high-titre anti-C1q antibodies.

We have therefore recently suggested that patients with SLE and persistently low complement levels be treated with prophylactic penicillin, and considered for pneumococcal and meningococcal vaccination [60], and this is now our unit policy.

It is worth noting that one of the four lupus patients who died succumbed not from a pneumococcal or neisserial infection, but with septicaemia due to Pseudomonas. Two research fellows working in our laboratory for brief periods under my supervision—Marco Tonoli from Verona, and Marc Seelen from Professor Daha's laboratory in Leiden—have recently been exploring the role of complement and both CR1 and CR3 in the processing of Gram-negative organisms in a range of in vitro assay systems. Dr Tonoli demonstrated that a monoclonal antibody HA-1A, used in the therapy of septic shock, could mediate complement fixation on red cells and binding to CR1 [61] and, more
recently, we have shown that complement, fixed by both alternative and classical pathways, can directly mediate binding of Gram-negative bacteria to leucocyte CR3 [62], as shown in Fig. 22. It should not be forgotten that one of the main physiological roles of the complement system is in defence against infection, and our results would suggest that in hypocomplementaemic states there will be increased susceptibility to both Gram-positive and Gram-negative infection.

Is there a role for repletion of complement proteins in lupus?

The second clinical question which we might consider relates to the use of complement proteins in the treatment of SLE. Should replacement therapy be considered in all deficient patients? Would it be appropriate to use regular therapy, say with FFP, in all patients who are chronically hypocomplementaemic with SLE? Is there a role perhaps for soluble CR1 in patients who have low levels on their erythrocytes? There are two major considerations to be taken into account when trying to answer these questions. (i) Do we know how often treatment should be given, and how much would be needed? (ii) Are there any potential risks to a therapeutic approach of this kind? Our Icelandic patient clearly benefited from treatment with FFP for some weeks, even though her classical pathway activity was normalized for only a few days. Most complement proteins turn over at ~1%/h, and infusion of sufficient FFP to normalize C3, or C4 concentrations, would only result in a similarly transient correction of the plasma level of the protein. In the C2-deficient case, we assume that correction of hypocomplementaemia may have resulted in mobilization of ICs from sites of inflammation, notably the skin, and facilitated their effective processing by the reticuloendothelial system. It is possible that it then takes some weeks for the further generation and deposition of ICs to occur and produce local inflammation. These mechanisms are poorly understood, and it would be very difficult to estimate how frequently therapy would be indicated in any one patient with SLE and genetic or acquired complement deficiency. To normalize classical pathway activity on a long-term basis requires treatment with many units of plasma every few days. It is unlikely that it would be commercially viable for a pharmaceutical company to produce a purified or recombinant protein to be used in the treatment specifically of complement-deficient patients, in view of the rarity of these patients. The regular use of the large quantities of FFP required would be both prohibitively expensive, and expose the patients to the risks of acquiring retroviral, hepatitis C or other infections. In addition, there are other immunologically important risks of such therapy. It should not be forgotten that complement, in addition to facilitating the processing of ICs, as we have demonstrated in the work described.

---

**Fig. 24.—**E-CR1 can protect the endothelium from PMN and IC-mediated damage (from Beynon et al. [66], with permission).
in this essay, also has a major role mediating the inflammatory process, both in autoimmune and infective diseases. Complement deposition is a characteristic feature of glomerulonephritis in SLE, and C3 products can readily be demonstrated in inflamed skin, or in the surface of erythrocytes in patients with SLE and haemolytic anaemia. There is good evidence from studies performed in genetically C3-deficient dogs with nephritis that the disease is exacerbated by plasma infusion, and the normalization of complement levels [63]. We have also demonstrated in humans that providing a missing complement component may have potentially adverse consequences. In a patient with hereditary C6 deficiency and meningococcal meningitis, we showed that infusion of FFP resulted in a clinical deterioration rather than an improvement, and that this was associated with a rise in endotoxin levels [64] (Fig. 23). This patient also developed antibodies to C6, which resulted in a less marked response to later infusions of plasma. We have also recently demonstrated the development of antibodies to the missing component in the serum in a C1q-deficient patient treated on multiple occasions with FFP. Subsequent therapy with plasma at a time of an exacerbation of his lupus resulted in the development of an acute serum sickness-like illness [59]. Clearly, the development of antibodies in this way might also reduce the efficacy of repeated therapy, as well as putting the patient at risk of further IC-mediated disease.

Should we consider the use of recombinant soluble CR1 (sCR1) in SLE? This compound has been used in a number of experimental models of inflammation (recently reviewed by Fearon and colleagues [65]), including cardiac allo- and xenografts, IC-mediated vasculitis, thermal trauma, and both myocardial and intestinal reperfusion systems. Early evidence suggests that sCR1 may have a role in reducing inflammation acutely, and in protecting grafts, and a trial of its use in acute myocardial infarction in humans is under way. We have recently published work which suggests that CR1 on erythrocytes may have a direct role in protecting the endothelium from IC and neutrophil-mediated damage [66]. In these studies, a model system was developed by Huw Beynon in our laboratory, in which he assessed endothelial damage by growing the cells directly on a fluorescein isothiocyanate (FITC) matrix, and measuring the effect of different potentially phlogistic stimuli on the access of a radiolabelled anti-FITC antibody [67]. An increase in permeability in this system equates with greater cell damage. Figure 24, taken from Dr Beynon's recent paper in the *Journal of Immunology*, demonstrates the protective effect of red cell CR1 on endothelial cells cultured in the presence of IL-1, neutrophils and ICs.

A detailed discussion of leucocyte–endothelial interactions in SLE is clearly beyond the scope of this essay, but we have reviewed the subject recently [68]. We suggest that one of the functions of CR1 may be to
'capture' ICs in the circulation, and keep them in the faster flowing central jet stream in the blood, away from the endothelial surface where, in association with neutrophils, they have the potential to cause damage (Fig. 25). Physiologically, it is erythrocyte-bound CR1 which subserve this function, but it is possible that sCR1, administered in sufficient dose, might be able to work in a similar way. The most likely clinical situation in which one might consider this approach is in the context of an acute inflammatory exacerbation of the condition. However, we do not know whether the soluble form of CR1 would be as effective as erythrocyte CR1 which, as we have discussed previously, is clustered, and has very high affinity for ICs, and many of the same questions which we raised in the context of repletion therapy for deficient plasma proteins—e.g. how much, and how often?—are difficult to answer. The data we obtained in the radioimmunotherapy patients suggested that CR1 may be rapidly proteolytically degraded when large numbers of ICs are presented to the fixed macrophage system, and if a similar effect were observed with sCR1, it is likely that it would only be of transient benefit. It is perhaps also worth sounding a note of caution regarding the extrapolation of observations made in animals to the human situation. There is a great deal of interest at present in the use of pig organs in xenotransplantation, and the possible role of sCR1 or other complement-inhibitory molecules in prolonging graft survival. We have recently studied the clearance of soluble model ICs (HBsAg: pig anti-HBsAg) in the pig (work reported at the recent European Complement Workshop in Switzerland [69]) and, to our surprise, we found that IC initially localized primarily to the lungs, rather than to the liver and spleen, as in man, and that this uptake was highly complement dependent, reflecting what would appear to be fundamentally different organization and function of the reticuloendothelial system in this species.

CONCLUDING REMARKS

On the basis of the work described in this essay, is it possible to formulate a unifying hypothesis to explain the role of complement and CR1 in pathological IC processing? An attempt is made in Fig. 26, in which we compare 'physiological' IC processing with the situation in SLE. We postulate that IC formation, either at sites of tissue injury or in the circulation, may cause complement activation and the deposition of C3 fragments on erythrocytes, and the subsequent clearance of

---

**Fig. 26.—Proposed schema for the immunopathogenesis of SLE.**

---
these IC may result in a fall in erythrocyte CR1. These abnormalities may then result in defective further processing of complexes, as a consequence of poor opsonization, failure of modification of IC structure and non-physiological delivery to the fixed macrophage system, as a result of impaired binding to CR1. Such defective processing may, in itself, result in the persistence of potentially harmful complexes, causing tissue damage, further systemic and local complement activation, and the development of a positive feedback loop stimulating further antigen release and autoantibody production. Data have also been presented that suggest that erythrocyte CR1 may have a specific role to play in the protection of the endothelium from direct neutrophil and IC-mediated damage, and the acquired fall in CR1 numbers observed in SLE would clearly result in a reduction in the efficacy of such a protective mechanism. We also suggest that hypocomplementemia, a reduction in erythrocyte CR1 and functional hyposplenism may predispose certain patients with SLE to infection. We are currently involved in exploring these hypotheses further, both in humans and in the pig, and in a mouse model of C1q deficiency, which is being developed by Marina Botto in our laboratory using gene ‘knockout’ technology.

It remains our hope that the development of a fuller understanding of the relationships between complement proteins and receptors, ICs, and the many other components of the cellular and humoral immune systems in SLE, will one day enable us to devise more rational and effective treatments for this most fascinating, but frustrating, rheumatic disease.

ACKNOWLEDGEMENTS

All the work described was supported by the Arthritis and Rheumatism Council, who remain most generous supporters of both myself and the RPMS Rheumatology Unit. I would like to acknowledge the guidance and support of Professor Mark Walport, and am also grateful to Dr Huw Beynon, Dr Jurg Schifferli, Dr John Savill, Dr Mike Peters, Dr Chris Bunn, Dr Sozos Loizou, Dr Dorian Haskard and Mr Peter Norworthy for their advice and practical assistance. Figures 6–14 appear with kind permission of the Editor at the Journal of Clinical Investigation, and Figures 2–5, 16–21 and 24 with kind permission of the Editor of the Journal of Immunology.

REFERENCES


