Immunobiology 224 (2019) 511–517

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

The story of complement factor I

Peter J Lachmann

Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, United Kingdom

ARTICLE INFO

Keywords:
Complement
Conglutinin
Factor I
C3b feedback cycle
C3b breakdown cycle
Age related degeneration

ABSTRACT

Factor I was first discovered in 1966. Its importance became apparent with the description of the original Factor I deficient patient in Boston in 1967. This patient presented with a hyperactive alternative complement pathway resulting in secondary complement deficiency due to continuous complement consumption. On the basis of these findings, the mechanism of the alternative pathway was worked out. In 1975, the surprise finding was made that elevating levels of Factor I in plasma down-regulated the alternative pathway. Attempts to exploit this finding for clinical use had a long and frustrating history and it was not until 2019 that the first patient was treated with the gene therapy vector for age related macular degeneration by Professor Sir Robert MacLaren in Oxford. This review follows the long and contorted course from initial observations to clinical use of complement Factor I.

1. The discovery of factor I

The molecule now known as Factor I was first discovered in 1966 from two quite separate sets of experiments. Tamura and Nelson (1967) studied complement inhibitors in guinea pig and rabbit serum. These included C1 inhibitor, which is well known (Levy and Lepow, 1959), an inhibitor of C6 which probably does not exist but could have been a C567 uptake inhibitor, such as vitronectin, and a C3 inhibitor which they showed to inhibit lysis and immune adherence. This molecule was probably Factor I but could also have been Factor H on the basis of purification data, or probably a mixture of the two. They did little further work on it. A molecule that was certainly Factor I was described a year later by Torisu et al (1968) who identified it as transferrin. This was incorrect but Factor I and transferrin do co-purify on ion exchange and size chromatography.

The other approach (Lachmann and Müller-Eberhard, 1967; 1968) identified Factor I as the Konglutinogen activating factor (KAF). This requires a discussion of conglutination, which follows in some detail.

2. Conglutination

The phenomenon of conglutination was described in several laboratories at the beginning of the twentieth century. The phenomenon as described was the powerful conglutination or clumping of antibody-coated red cells when treated with a non-lytic complement, usually provided by horse serum when using sheep EA[1] . This tight clumping was produced by heated bovine serum and the phenomenon was given the name “conglutination” by Bordet and Streng (1909). Towards the beginning of the Second World War Robin Coombs, who had just qualified as a veterinary surgeon in Edinburgh, was sent to the Veterinary Research Laboratory of the Department of Agriculture in Weybridge to work with Norman Hole on a conglutinating complement fixation test for glanders in horses. Rather surprisingly glanders in horses was considered to be relevant to the war effort at the beginning of World War Two. While that turned out to be a misconception, Robin Coombs did get extremely interested in complement fixation and conglutination and was transferred to Cambridge to do a PhD. While in Cambridge, he got involved with Robert Race and Arthur Mourant in the development of the Coombs Test or antiglobulin reaction (Coombs et al., 1945) but his PhD thesis contained a lot of work on conglutination as well. This interest he continued for some years after the war in conjunction with Ann Blomfield (later Ann Coombs) and a Canadian co-worker Donald Ingram. They distinguished conglutinin from immuno-conglutinins which are formed in response to stimulation by fixed complement and looked at their role in disease. This work was published as a book “Serology of conglutination” (Coombs et al., 1961).

I returned to the Department of Pathology in Cambridge from a year in New York where I had first worked together with Hans Müller-Eberhard and Henry Kunkel on the localisation of bound C3 in human tissues (Lachmann et al., 1962) using the first antibody raised to a specific complement component by Müller-Eberhard and his colleagues while he worked in Sweden (Müller-Eberhard et al., 1960).

In Cambridge I undertook to work on conglutinin. It turned out that the reaction of conglutinin with zymosan, which was well known, occurred equally well in the absence of complement, i.e.it could be taken up from heated bovine serum. This reaction required calcium ions and

E-mail address: pjl1000@cam.ac.uk.

[1] EA is short for antibody sensitised sheep red cells.

https://doi.org/10.1016/j.imbio.2019.05.003
Received 3 April 2019; Received in revised form 8 May 2019; Accepted 8 May 2019
Available online 10 May 2019

was readily reversible with EDTA. This allowed a thousand-fold purification of conglutinin simply by absorption onto zymosan, elution with EDTA and precipitation as euglobulin. This reaction of a pure carbohydrate in the presence of calcium defines a C-type lectin and conglutinin was actually the first mammalian example of this class of molecule to be described, shortly before mannan binding protein. Conglutinin was found to be a molecule of high asymmetry with a sedimentation coefficient of 7.75 and a frictional ratio of 4.0 (Lachmann and Coombs, 1965). From these data a molecular weight of 746,000 was calculated. Conglutinin does indeed only occur in some bovidae - cows, camels and llamas, but not in sheep and goats. The claim that there was a human conglutinin (Baatrup et al., 1987) was disproved finally through the absence of a functional conglutinin gene within the human genome sequence.

Having identified conglutinin, the question then became what does it react with?

3. The identification of the conglutinogen

Conglutinin was shown to not to react with EA treated with guinea pig R3 (serum treated with zymosan) but did react when human R3 was used. It was already known that the former reagent was EAC142[2] and the latter was EAC1423 and reacted with anti-β1c. Conglutinin therefore appeared to react with bound β1c. β1c was the name given to the protein identified by immunoelectrophoresis that was identified as C'3.

In La Jolla in 1966, I undertook the study of where the conglutinogen lay using the purified components available in the Müller-Eberhard laboratory. It became clear that EAC1423 made with purified components did not react with conglutinin but if this intermediate was treated with 56 °C heated normal serum, it rapidly became conglutinatable. Using this assay, Factor I (at that time called KAF – Konglutinogen Activating Factor) (Lachmann and Müller-Eberhard, 1968) was purified from human serum. It was found to be a beta-globulin protein which fractionated on both ion exchange chromatography and size exclusion chromatography together with transferrin. This separation of Factor I from transferrin required a further step of hydroxyapatite chromatography which then allowed the protein to be substantially pure. Specific polyclonal antibodies could be raised against it and it could also be purified from antibody columns. KAF was shown not to be consumed in its reaction with bound C3b. Therefore, it was concluded that KAF was an enzyme, which in due course was confirmed when it was later found that a small peptide, C3f, was released from C3b (Harrison and Lachmann, 1980) when it is converted to iC3b - the intermediate that reacts with conglutinin. iC3b therefore presents a carbohydrate neoantigen that is hidden in C3 and in C3b. This neoantigen could be destroyed by fungal "cellulase" (from Worthington Biochemicals) and occurs in C3 of all mammalian species tested (Lachmann and Coombs, 1965).

In 1966, Ulf Nilsson had suggested to me that we should try to see whether his recently identified protein β1H (Nilsson and Mueller-Eberhard, 1965) played some part in the conglutination reaction. This was tested but no effect was found. This failure to demonstrate the effect of β1H globulin (now called Factor H) on conglutination was due in retrospect to the fact that the C3 preparations were contaminated with small amounts of β1H, which co-purifies with C3 rather closely (Nilsson and Mueller-Eberhard, 1965). We therefore failed to make the discovery that Factor I requires Factor H as a co-factor which was subsequently made ten years later by Whaley and Ruddy (1976) and revolutionised our knowledge of how Factor I actually worked. The mechanism was elegantly worked out using modern techniques of structural biology by Roversi et al (2011) as shown in Figs. 1 and 2.

The conglutinogen in bound iC3b was found to be apparently identical with the determinants found in yeast, which was remarkable.

[2] EAC is short for EA treated with complement.

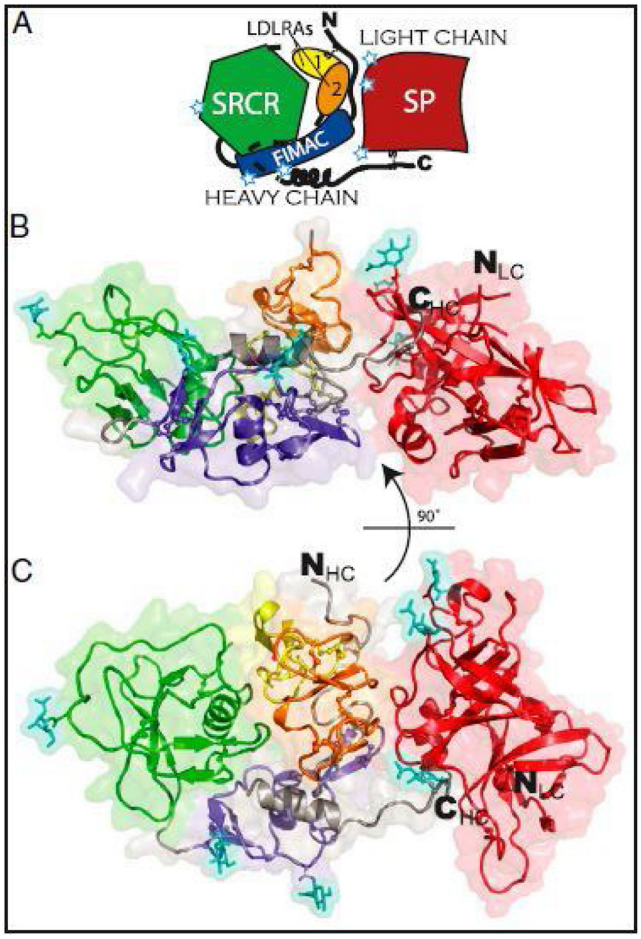

Fig. 1. The structure of human Factor I. (A) Cartoon schematic of Factor I. FIMAC is shown in blue, SRCR in green, LDLRA1 in yellow, LDLRA2 in orange, and SP in red; unmodeled loops are shown by dashed lines; N-linked glycosylation sites are shown by white stars. (B and C) The protein is shown in two views as a cartoon representation with a transparent surface. Disulfide bonds and two bound Ca2+ ions are shown as ball-and-stick representations. The six glycosylated Asn residues and attached GlcNAc residues (cyan) are shown as stick representations. Domains are coloured as A (Roversi et al., 2011) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In a direct carbohydrate binding assay conglutinin was shown to bind non-reducing terminal N-acetylglucosamine, mannose and fucose residues (Loveless et al., 1989).

4. Factor I deficiency and the mechanism of action of the alternative complement pathway

In 1967, Chester Alper and colleagues (Alper et al., 1970) first identified a patient (TJ) who had Klinefelter's syndrome and repeated infections and who was found to have a grossly abnormal complement system and very low levels of C3 and of the protein that is now known as Factor B, both of which occurred in cleaved form. The C3 was what is now known as C3b and the Factor B was circulating as Bb. Chester had no explanation for these findings and when we met at the Protides of the Biological Fluids meeting in 1969 in Bruges, he described this patient to me and I suggested to him, without any real prior justification, that it was just possible that this patient might lack KAF, or Factor I. This was rapidly shown to be the case when we sent Chester some anti-Factor I antibody. Not that this immediately clarified what was going on. However, we did do an experiment on TJ, injecting him with purified Factor I, which did restore temporarily the C3 and Factor B levels

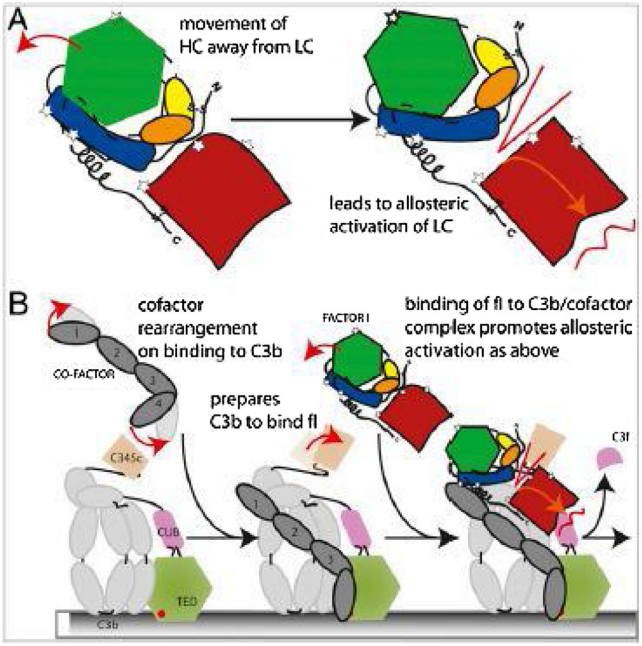

Fig. 2. Schematic of the proposed cofactor model. Red arrows indicate domain rearrangements. (A) Cartoon of the proposed allosteric activation of the SP domain via alteration of the heavy-chain (HC)/light-chain (LC) interface. (B) Schematic of the assembly of the C3b:fH1–4:fI ternary complex, colored as in Fig. 1, extended from cartoon of cofactor–C3b interactions (Roversi et al., 2011) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

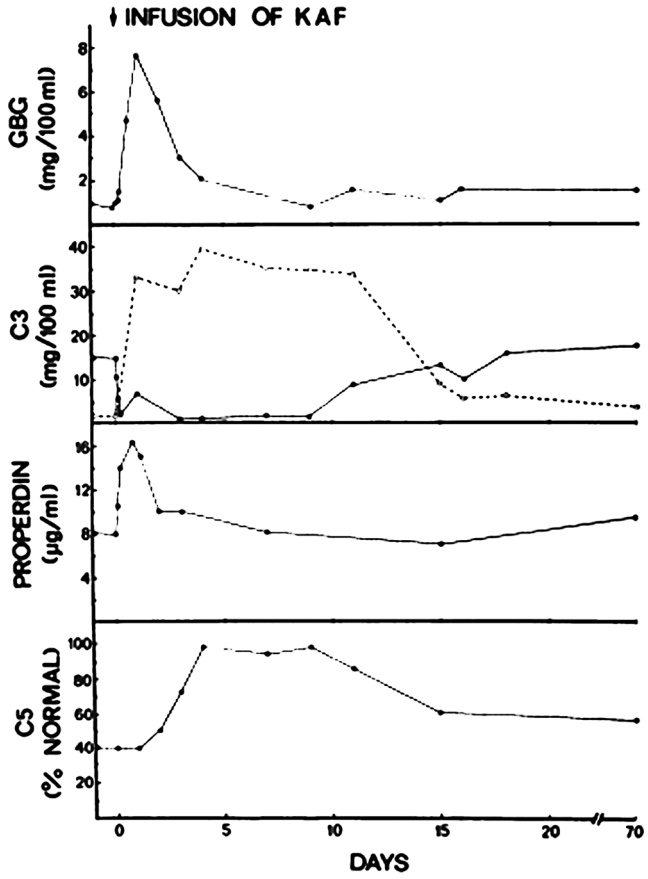

Fig. 3. Infusion of Factor I into deficient patient.
Immunochemically determined serum protein concentrations. In the graph representing C3 concentration, open circles represent native C3 and closed circles represent conversion products (usually C3b). Normal ranges are: Factor B (GBG), 12–56 mg/100 ml; C3, 100–200 mg/100 ml; properdin, 8–30 µg/ml; C5, 41–158% normal (from Ziegler et al., 1975).

(Ziegler et al., 1975) (Fig. 3).

It was not a wholly satisfactory experiment since we sent Factor I that had been purified and stored and was sterilised by filtration, but we had not thought of clearing it of endotoxin. The result was that TJ had an endotoxin reaction and rigors but he came to no serious harm (Ziegler et al., 1975). It did demonstrate that his complement abnormalities were in fact due to deficiency of Factor I. I then, in Cambridge and subsequently at the Royal Postgraduate Medical School, undertook to repeat this phenomenon in vitro with my graduate student, Pru Nicol (Nicol and Lachmann, 1973; Lachmann and Nicol, 1973a). We depleted normal human serum of Factor I using the purified polyclonal Fab_2 fragments against Factor I. This was made by a technique developed shortly before (Lachmann, 1971) where a precipitate is made between a monospecific antibody and the antigen, either purified or in serum. The precipitate is then washed free of all other components, redissolved in glycine/HCl at pH 3.2, and treated with pepsin. This destroys most protein antigens except the Fab_2 part of IgG so that this releases in solution the Fab_2 portion of the original antibody, which can be precipitated with sodium sulphate. When this purified Fab_2 was used to deplete KAF at 4 °C, the precipitate was removed and at 4 °C nothing further happened. However, immediately the serum was heated up there was total conversion of C3 to C3b, and Factor B to Bb and Ba, just as was seen in TJ (Nicol and Lachmann, 1973). This we interpreted as demonstrating that what is known as the alternative complement pathway did not in fact require specific initiation but would fire spontaneously as soon as an inhibitor controlling it was removed, suggesting that there was a continuous balance between a breakdown cycle of C3b, produced by Factor I, and an amplification loop which was already known where C3b combines with Factor B to give rise to C3bBb, which is then cleaved by Factor D to give rise to a C3 convertase. This kinetic scheme of the alternative pathway was subsequently refined to take account of the role of Factor H as a cofactor for Factor I, but essentially has stood the test of time. What is required is that there must always be a trivial amount of C3 activation in progress so that the two cycles are kept in balance and so that the removal of Factor I instantaneously activates the pathway. This was known as the “tickover hypothesis” the analogy being with an engine which ticks over but the car doesn’t move, but if then the cycle is accelerated the car can be propelled forwards (Lachmann and Nicol, 1974; Lachmann and Halbwachs, 1975). The nature of the tickover was not then, or even now, securely known, but Pangburn and colleagues (1981) made the suggestion that the water hydrolysis of C3 at a very low rate might provide the trigger amount of a C3b-like molecule known to keep the tickover functioning. This idea is plausible and attractive but is almost impossible to test. Unfortunately it has been quite widely misunderstood and recent papers on the tickover (Nilsson and Nilsson Ekdahl, 2012) postulate the formation of substantial amounts of a tickover component which is clearly iC3i and cannot be the molecule that produces the tickover. It is quite likely there are multiple mechanisms for maintaining tickover. The water hydrolysis is one, but trivial amounts of C3b may also be produced by other enzymes, either from the clotting system, plasmin or neutrophil elastase. The classical and lectin pathways are likely to be activated quite frequently and traces of C4b2a or of MASP-2 (which can directly cleave C3) will provide more than enough C3b for tickover (Yaseen et al., 2017). It is only when reactions are performed in Mg EGTA to remove calcium that the alternative pathway can be fired in isolation. However, even then caution is needed since one group of lectin pathway activators, the ficolins, are not C-type lectins and do not need calcium for activation of the lectin pathway. They may be involved in some reactions which are currently regarded as exclusively due to the alterative pathway.

Table 1
S77 prevents the complement activation produced by Factor I depletion of serum.

S77 concentration μg/ml	422	211	105	53	26	13	0
% haemolysis	95	95	95	95	95	0	0

Recently it has become possible to confirm the tickover hypothesis by the use of an antibody (S77) that inhibits it. This antibody was made by Genentech (Katschke et al., 2009) and is directed against a determinant on the MG7 domain of the C3 alpha chain. It reacts with C3b, iC3b and C3c but not with native C3; and inhibits C3b binding to Factor B, Factor H, CR1 and C5, but not to properdin. This antibody, we were able to show, inhibited tickover (Lachmann et al., 2018a) when tested in the system which gave rise to the concept of "tickover", i.e.activation of the alternative pathway by Factor I depletion of serum. S77 to a concentration of about 26 μg/ml can prevent this activation (Table 1).

S77 dilutions in veronal/EDTA were added to 50% NHS and incubated at 4 °C for 30 min.. Fab_2 anti-factor I was added and incubated on ice for 1 h. The precipitate was spun down and the supernatant removed. Rabbit erythrocytes and Mg were added, incubated at 37 °C and haemolysis read.

It can also be shown directly to inhibit the binding of C3b to Factor B, which is the essential reaction for tickover. Interestingly, this inhibition is markedly suppressed if nickel is used instead of magnesium. It is known that nickel greatly increases the affinity of Factor B for C3b (Fishelson et al., 1983) and this, therefore, is evidence that this is the reaction which is inhibited. Exactly how S77 produces these effects is not clear as, for example, it reacts also with C3c which is not known to bind Factor B. The antibody might be useful in treating PNH but for other purposes the fact that it can react with so many C3 breakdown products means that it is likely to be consumed where there is ongoing complement activation by other pathways.

The intriguing example that springs to mind concerns the original experiments of Pillemer et al (1954) who isolated properdin by treating serum with zymosan at 17 °C and then eluting the properdin with a slightly alkaline, high ionic strength buffer. This eluted material needed to be added back to a mixture of zymosan and properdin-depleted serum for the inactivation of "C'3″ to occur above 20 °C. The removal of properdin by zymosan appeared to need components of the classical pathway but the reaction was done in serum treated with a calcium binding resin so that calcium should have been absent. This would suggest (reviewed in Lachmann, 2018) that C3b would become bound to zymosan as a result of lectin pathway activation through ficolins which do not require calcium and, being on a protected surface, would preferentially bind Factor B rather than Factor H. While formation of the active alternative pathway C3 convertase requires a temperature around 37 °C, these experiments suggest that Factor B does bind to C3b at 17 °C and allow properdin to bind to the complex.

The description of the alternative pathway as having no initiating event, but being entirely rate-governed by the balance between the C3b feedback cycle and the C3b breakdown cycle was reported at the International Complement Meeting on Coronado Island in 1973 (Lachmann and Nicol, 1973b). The presentation had a mixed reception. In particular it was rejected by the La Jolla group who were at that time, and for some years afterwards, wedded to the idea that there was an "initiating factor "for the alternative pathway which was non-immunoglobulin in nature and was the physiological analogue of the C3 nephritic factors that were described originally by Spitzer and colleagues (1969) and which Thompson (1972) had shown to be associated particularly with IgG3. The papers by Schreiber et al on the initiating factor were published in 1976 (Schreiber et al., 1976a,b) and have never been withdrawn. It is curious, and perhaps ironic, that in retrospect it was realised by Dr. Schreiber (personal communication) that the initiating factor that they had purified was in fact Factor I. What is less clear is why they thought that this was a method for initiating the alternative pathway. It was also subsequently shown with unimpeachable clarity that nephritic factors are indeed immunoglobulins. Davis and his colleagues (1977) showed that they cross the placenta, which is peculiar to IgG among molecules of that size, and the Hammersmith group (Scott et al., 1978, 1981) showed that nephritic factors could be converted to Fab2 or Fab fragments, and that these were still active. This group also subsequently showed that the peculiar activity of these antibodies, which were somewhat larger than normal IgG, seemed to be due to an abnormal content of carbohydrate since treatment with periodate stopped them from stabilising the C3 convertase and converting C3 in normal serum.

An unexpected property of Factor I was described in 1975 (Lachmann and Halbwachs, 1975) who did experiments increasing the Factor I concentration in serum and looking at what effect this had on alternative pathway activation. It was shown that raising the Factor I concentration markedly enhanced the C3b breakdown cycle and inhibited the alternative pathway quite strikingly. Increasing the concentration by only about 25 percent almost completely inhibited alternative pathway activation by particulate inulin or LPS. This was surprising then and remains surprising now because in general, in the triggered enzyme cascades of blood plasma, the enzymes are present in considerable excess of the amount required and are not rate limiting. In the case of the alternative pathway, this is not true and a similar phenomenon is true of Factor D where increasing concentrations have a potentiating effect, certainly on lysis produced by the alternative pathway, as shown by Mathieson et al (1993) who demonstrated that it is the regions of adipose tissue that have high concentrations of Factor D that are affected by partial lipodystrophy in patients with nephritic factors who suffer from this disease.

It was recognised almost at once that this property of Factor I gave rise to a promising therapeutic for inflammation where the alternative pathway plays an important role. In the mid-1980s Cell Tech Ltd., which was a company devoted to bio-therapeutics, agreed to make some Factor I which might be tested – at that time it was thought possibly in rheumatoid arthritis and/or systemic lupus. They did indeed make a substantial quantity of human Factor I by affinity chromatography using the MRCOX21 monoclonal anti-Factor I antibody (Sim et al., 1993) by the method of Hsiung et al. (1982). Unfortunately, on analysis, it turned out that this antibody preparation contained quantities of mouse DNA in excess of that allowed by the regulatory authorities. Although this is almost certainly of no clinical significance, it prevented this antibody being used in vivo and Cell Tech Ltd., rather than admitting that they had made a mess of the preparation by not cleaning up their monoclonal antibody sufficiently and then making another batch, simply dropped the project. This was perhaps my first introduction to the well-known policy in the biotech industry of "fast to fail". This involves taking the idea and doing one single experiment to try to take it to the clinic as quickly as possible without necessarily doing the prior studies that are needed in detail. If this fails, they drop it, and if it succeeds they then charge enough for it to recompense themselves for all the examples that have failed. This common, but wholly deplorable practice, is one of the factors that leads to medicines being so prohibitively expensive. Nevertheless, involvement of Cell Tech Ltd. Accelerated the cloning and sequencing of Factor I (cDNA and derived amino acid sequence) (Catterall et al., 1987).

5. The actions of factor I

The original activity of Factor I was to convert C3b to iC3b (Fig. 4).

For this purpose it requires a co-factor which in the fluid phase is Factor H but on cells can be MCP (CD46) (Seya et al., 1986) or CR1 (CD35) (Medof et al., 1982). Factor I has, however, an extremely important further physiological activity. This is to convert iC3b to C3c and C3dg. For this purpose, it cannot, under physiological conditions, use Factor H as a co-factor. This is because the affinity of Factor H for iC3b is very low. Were this not the case it would clearly compromise the C3b

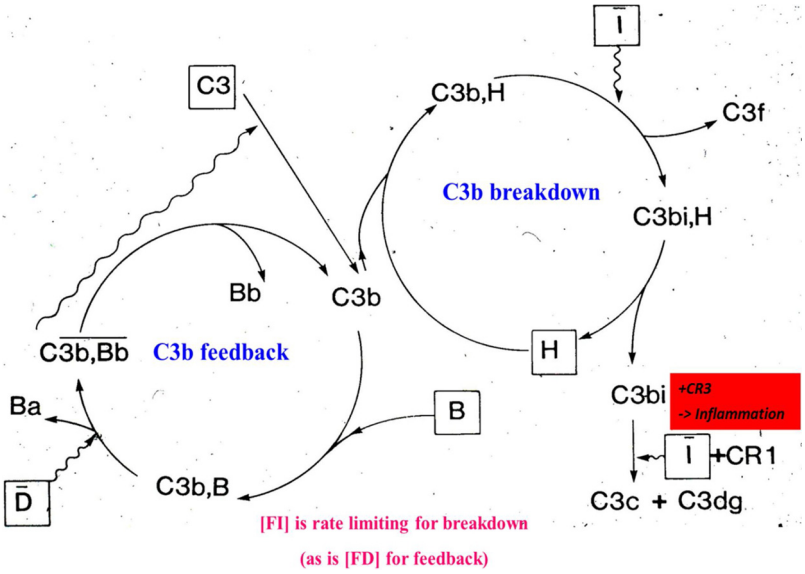

Fig. 4. C3b Feedback and Breakdown Cycles.

breakdown loop since it is necessary to release the Factor H from iC3b so that it can react with further C3b. In vivo, the co-factor that is required for the cleavage of iC3b to C3c and C3dg is CR1. In humans, this receptor is found only on red blood cells in the circulation but it is also present on neutrophils and monocytes when these cells are on surfaces. For experimental purposes, however, it is possible to use Factor H as a co-factor for this final split simply by reducing the ionic strength (Lachmann et al., 2018a,b). This is convenient since it is easy to make EAC3bi simply by treating EA with a human R3 reagent (human serum absorbed with zymosan). EAC3bi reacts with conglutinin and the reactivity is lost by the cleavage of iC3b which elutes C3c and leaves C3dg on the red cell surface. This in-vitro test demonstrates quite conclusively that it is only the affinity of iC3b for its co-factors that is important in their activity.

The reason that this final split is so important in vivo is that a principal and necessary mechanism for much complement mediated inflammation is the reaction of iC3b with the complement receptors CR3 (and probably CR4 as well) on neutrophils. In the absence of iC3b complement mediated immunopathology is not seen. This was extremely elegantly demonstrated in mice by Rose et al (2008) who demonstrated that glomerular damage occurs spontaneously in Factor H knockout mice but not in Factor I knockout mice. They then made double knockouts of both Factor H and Factor I and they again failed to show immunopathology. When these mice were injected with normal (Factor I-containing) mouse serum the renal immunopathology appears within hours demonstrating conclusively the need for Factor I in generating iC3b and causing immunopathology. An observation analogous to what was found in the mice was already known in humans where totally Factor H-deficient subjects (reviewed by Pickering and Cook, 2008) and a whole population of Factor H-deficient pigs (Høgåsen et al., 1995) suffered from severe renal disease, whereas Factor I deficient subjects may have pyococcal infections as did the original patient, TJ, or may have just the occasional meningococcal infection, as did our second patient in the North of England (Thompson and Lachmann, 1977) and occasionally they may even be normal. There are now at least thirty reported homozygous Factor I deficiencies and their clinical status is reviewed by Alba-Domínguez et al. (2012). Heterozygous Factor I deficiencies are much commoner and comprise 7.8 per cent of cases with age related macular degeneration (AMD) (Kavanagh et al., 2015). Heterozygous Factor I deficiency is also a susceptibility gene for atypical haemolytic uraemic syndrome (Fremeaux-Bacchi et al., 2004). The very rare occurrence of renal disease in these patients may be an ascertainment artefact since it is patients with renal disease who have their complement measured, but it is also possible, and there is a reported case (Sadallah et al., 1999), that this is the consequence of bacterial infection following on the Factor I deficiency giving rise to immune complex glomerulonephritis.

The major immunopathological consequences of the hyperactive alternative pathway are the renal diseases (atypical haemolytic uraemic syndrome, dense deposit disease and C3 glomerulopathy) and age related macular degeneration. The two are associated and the patients who get retinal changes earlier in life than anyone else are the patients with nephritic factors (Duval-Young et al., 1989; Colville et al., 2003). It is therefore extremely likely that the pathogenesis is at least in part similar and that the changes giving rise to the macular degeneration occur over a much longer period. These may result from repeated Gram negative infections or exposure to endotoxin over a long period of time. Exacerbation of the disease by smoking is a further indication that polymorphs are likely to be involved. The nephritic factor positive patients also show that the complement mediated (chromosome 1-associated) age related macular degeneration is a systemic disease (Scholl et al., 2007).

Factor I deficiency, therefore, turns out to be a very strange condition. Heterozygous Factor I deficiency where the levels are low has the highest penetrance of any complement abnormality in age related macular degeneration (Kavanagh et al., 2015) and as far as immunopathology is concerned it is the heterozygotes that are at risk whereas the homozygotes are probably completely protected.

6. The therapeutic use of factor I

With the advent of knowledge about age related macular degeneration, the attitude of the pharmaceutical industry to the complement system in general and to Factor I changed since they now had an immensely common disease where there was a major unmet medical need. The potential use of Factor I as a rational way of down-regulating the alternative complement pathway is persuasive. Since this involves only increasing the concentration of a normal plasma component there is no likelihood of immunogenicity. The plasma concentration of Factor I is fairly low (around 35 μg/ml) so that effective elevation of concentration requires much less protein than would be the case with Factor H (whose plasma concentration is around 500 μg/ml. Raised Factor I levels are further capable of accelerating the break down pre-existing iC3b (a property not shared by Factor H). A further advantage is that raising

Factor I concentration provides a titratable depression of complement activity rather than its total abolition. In spite of this it has proved extremely difficult to take this obvious therapeutic to the clinic.

The first logical way would be to prepare Factor I from plasma. Factor I is largely found in Cohn Fraction IV of plasma, which is generally discarded in plasma fractionation and of which large quantities therefore exist. There is one company, Kamada, in Israel that does have a license for fractionating Cohn Fraction IV and does make transferrin from it. Since this transferrin is made by ion exchange and size chromatography, it co-purifies with Factor I and their preparation of transferrin is very rich in Factor I (personal observations). Sadly, however, this material is not made to GMP because there is no clinical use for transferrin and it is made only for tissue culture. Although in practice this almost certainly makes no difference to its safety, the regulators are highly emphatic that it cannot be used even on human volunteers. Factor I purification from plasma would require de novo fractionation from Cohn Fraction IV and no plasma company has so far been willing to invest in this project. This leaves the alternatives of making Factor I by recombinant techniques or of using gene therapy.

The former was explored by my laboratory in conjunction with and supported by GlaxoSmithKline (GSK). The preclinical experiments were very encouraging (Lay et al., 2015; Lachmann et al., 2016) and recombinant Factor I could be generated by GSK at small scale. However, the scientific team were disappointed that scaling up production of recombinant Factor I, with the intent to manufacture, proved particularly challenging with extensive protein degradation and the company felt it was unlikely that it could be developed commercially. GSK deprioritized the project and soon after gave up ophthalmology altogether. When initial meetings to discuss the project were held there was considerable scientific enthusiasm for the project (which resulted in ˜4 years of collaborations with my laboratory and the initiation of a programme at GSK). However, knowing the challenges with scale up, there was a brief intervention by a lady from the commercial part of the company who told the assembled audience that if they imagined that GSK would commit to the large scare preparation of any protein other than an antibody they were fooling themselves and she then left. None of the rest of the audience took her warning sufficiently seriously but she was, of course, proved right and at the point when committing to large scale production had to be made GSK dropped the project.

Gene therapy is currently being explored. In mice, experimental gene therapy using an AAV8 vector for Factor I production in the liver has been very successful (Ahmad et al., 2017) and disease model testing is being undertaken. AAV8 delivery to human liver has so far proved much more difficult but it is hoped that this problem will be solved before too long. In the meantime, the local administration of a Factor I gene therapy vector to the eye is also under investigation. Ocular gene therapy has been effective in getting the therapeutic transgene expressed for several years, in a rare inherited blinding disease, (Leber Congenital Amaurosis type 2). The Factor I gene therapy investigation in the eye started in 2016, when a UK based start-up - Gyroscope Therapeutics – in-licensed from Cambridge University the intellectual property around the potential use of Factor I to restore the balance of the alternative pathway in AMD. A Factor I viral vector was developed and obtained all necessary regulatory approvals enabling further testing in AMD patients. In January 2019 Professor Robert MacLaren in Oxford treated the first patient with this vector, as part of a Gyroscope Therapeutics sponsored phase I-II clinical trial (NCT 03,846,193). Results are expected in 2021.

It is now forty-four years now since the publication of the paper by Lachmann and Halbwachs (1975) which underlies this therapy, and the first clinical trial was initiated in January 2019. It is a sad state of affairs that this has taken quite so long!

Conflicts of interest

No conflict of interest. In the past PJL has been a consultant to GSK and to Gyroscope Therapeutics.

Funding

This review did not receive any specific grant from funding agencies in the public, commercial, or non-for-profit sectors.

Acknowledgment

The author would like to thank Barbara Fernie-King for her invaluable assistance in the preparation of this manuscript.

References

Ahmad, A., Logan, G.J., Buchberger, A., Tay, S., Lachmann, P.J., Alexander, I.E., 2017. Modulation of Immune Responses Through Complement Down-regulation Using Adeno-associated Virus Mediated Gene Therapy. Children's Medical Research Institute, University of Sydney, Sydney, Australia (in-house publication).

Alba-Domínguez, M., López-Lera, A., Garrido, S., Nozal, P., González-Granado, I., Melero, J., Soler-Palacin, P., Cámara, C., López-Trascasa, M., 2012. Complement factor I deficiency: a not so rare immune defect. Characterization of new mutations and the first large gene deletion. Orphanet J. Rare Dis. 7, 42. https://doi.org/10.1186/1750-1172-7-42.

Alper, C.A., Abramson, N., Johnston Jr, R.B., Jandl, J.H., Rosen, F.R., 1970. Studies in vivo and in vitro on an abnormality in the metalolism of C3 in a patient with increased susceptibility to infection. J. Clin. Invest. 49, 1975–1985.

Baatrup, G., Thiel, S., Isager, H., Svehag, S.E., Jensenius, J.C., 1987. Demonstration in human plasma of a lectin activity analogous to that of bovine conglutinin. Scand. J. Immunol. 26, 355–361.

Bordet, J., Streng, O., 1909. Les phénomènes d'adsorption et la conglutinine du serum de boeuf. Zbl Bakt 49, 260–276.

Catterall, C.F., Lyons, A., Sim, R.B., Day, A.J., Harris, T.J., 1987. Characterization of primary amino acid sequence of human complement control protein factor I from an analysis of cDNA clones. Biochem. J. 242 (March (3)), 849–856.

Colville, D., Guymer, R., Sinclair, R.A., Savidge, J., 2003. Visual impairment caused by retinal abnormalities in mesangiocapillary (membranoproliferative) glomerulonephritis type II "Dense Deposit Disease". Am. J. Kidney Dis. 42 (2), E3.

Coombs, R.A., Mourant, A.E., Race, R.R., 1945. A new test for the detection of weak and incomplete Rh agglutinins. Br. J. Exp. Pathol. 26, 255–266.

Coombs, R.A., Coombs, A.M., Ingram, D.G., 1961. The Serology of Conglutination and Its Relation to Disease. Blackwell, Oxford.

Davis III, A.E., Arnaout, M.A., Alper, C.A., Rosen, F.S., 1977. Transfer of C3 nephritic factor from mother to fetus. Is C3 nephritic factor IgG? New England J. Med. Surg. Collat. Branches Sci. 297 (3), 144–145.

Duval-Young, J., MacDonald, M.K., McKechnie, N.M., 1989. Fundus changes in (type II) mesangiocapillary glomerulonephritis simulating drusen: a histopathological report. Br. J. Ophthalmol. 73 (4), 297–302 S.

Fishelson, Z., Pangburn, M.K., Müller-Eberhard, H.J., 1983. C3 convertase of the alternative complement pathway. Demonstration of an active, stable C3b, Bb (Ni) complex. J. Biol. Chem. 258 (June (12)), 7411–7415.

Fremeaux-Bacchi, V., Dragon-Durey, M.-A., Blouin, J., Vigneau, C., Kuypers, D., Boudailliez, B., Loirat, C., Rondeau, E., Fridman, W.H., 2004. Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. J. Med. Genet. 41, e84.

Harrison, R.A., Lachmann, P.J., 1980. Novel cleavage products of the third component of human complement. Mol. Immunol. 17, 219–228.

Høgåsen, K., Jansen, J.H., Mollnes, T.E., Hovdenes, J., Harboe, M., 1995. Hereditary porcine membranoproliferative glomerulonephritis type II is caused by factor H deficiency. J. Clin. Invest. 95 (3), 1054–1061. https://doi.org/10.1172/JCI117751.

Hsiung, L., Barclay, A.N., Brandon, M.R., Sim, E., Porter, R.R., 1982. Purificatioj of human C3b inactivator by monoclonal-antibody affinity chromatography. Biochem. J. 203, 293–298.

Katschke jr., K.J., Stawicki, S., Yun, J., Steffek, M., Xi, H., Sturgeon, L., Hass, P.E., Loyet, K.M., DeForge, L., Wu, Y., van Lookeren Campagne, M., 2009. Structural and functional analysis of a C3b-specific antibody that selectively inhibits the alternative pathway of complement. J. Biol. Chem. 284 (16), 10473–10479.

Kavanagh, D., Schramm, E.C., Triebwasser, M., Wagner, E.K., Raychaudhuri, S., Daly, M.J., Atkinson, J.P., Seddon, J.M., 2015. Rare genetic variants in the CFI gene are associated with advanced age-related macular degeneration and commonly result in reduced serum factor I levels. Hum. Mol. Genet. 24 (13), 3861–3870.

Lachmann, P.J., 1971. The purification of specific antibody as $F(ab')_2$ by the pepsin digestion of antigen-antibody precipitates, and its application to immunoglobulin and complement antigens. Immunochem 8, 81–88.

Lachmann, P.J., 2018. Looking back on the alternative complement pathway. Immunobiology 223, 519–523.

Lachmann, P.J., Coombs, R.R.A., 1965. In: Wolstenholme, G.E.W., Knight, J.J., Churchill, A. (Eds.), Complement, conglutinin and immunoconglutinins. Complement (Ciba Foundation Symposium), London, pp. 242.

Lachmann, P.J., Halbwachs, L., 1975. The influence of C3b inactivator (KAF) concentration on the ability of serum to support complement activation. Clin. Exp. Immunol. 21, 109–114.

Lachmann, P.J., Müller-Eberhard, H.J., 1967. The conglutinogen activating factor.

Protides Biol. Fluids 15, 469.
Lachmann, P.J., Müller-Eberhard, H.J., 1968. The demonstration in human serum of "conglutinogen activating factor" and its effect on the third component of complement. J. Immunol. 100, 691–698.
Lachmann, P.J., Nicol, P.A.E., 1973a. Reaction mechanism of the alternate pathway of complement fixation. Lancet 1, 465–467.
Lachmann, P.J., Nicol, P.A.E., 1973b. The C3 feedback cycle of complement activation. The role of the C3b inactivator (KAF). Proc Fifth Int Complement Workshop 111, 22289 (Abstr).
Lachmann, P.J., Nicol, P.A.E., 1974. Studies on the C3b feedback cycle. In: In: Raspe, G. (Ed.), Immunopathology VI Adv Biosciences Vol. 12. Pergamon Press, pp. 262.
Lachmann, P.J., Müller-Eberhard, H.J., Kunkel, H.G., Paronetto, F., 1962. The localisation of *in vivo* bound complement in tissue sections. J. Exp. Med. 115, 63–82.
Lachmann, P.J., Lay, E., Seilly, D.J., Buchberger, A., Schwaeble, W., Khadake, J., 2016. Further studies of the down-regulation by Factor I of the C3b feedback cycle using endotoxin as a soluble activator and red cells as a source of CR1 on sera of different complotype. Clin. Exp. Immunol. 183, 150–156.
Lachmann, P.J., Lay, E., Seilly, D.J., 2018a. Experimental confirmation of the C3 tickover hypothesis by studies with an Ab (S77) that inhibits tickover in whole serum. FASEB J. 32 (January (1)), 123–129.
Lachmann, P.J., Lay, E., Seilly, D.J., 2018b. A novel, sensitive and simple functional assay for Factor I based on the third proteolytic clip of C3b. J. Immunol. Methods 457, 30–32.
Lay, E., Nutland, S., Smith, J.E., Hiles, I., Smith, R.A.G., Seilly, D.J., Buchberger, A., Schwaeble, W., Lachmann, P.J., 2015. Complotype affects the extent of down-regulation by Factor I of the C3b feedback cycle in vitro. Clin. Exp. Immunol. 181 (2), 314–322.
Levy, L.R., Lepow, I.H., 1959. Assay and properties of serum inhibitor of C'1 esterase. Proc. Soc. Exp. Biol. Med. 101, 608–611.
Loveless, R.W., Feizi, T., Childs, R.A., Mizuochi, T., Stoll, M.S., Oldroyd, R.G., Lachmann, P.J., 1989. Bovine serum conglutinin is a lectin which binds non-reducing terminal N-acetylglucosamine, mannose and fusose residues. Biochem. J. 258 (1), 109–113.
Mathieson, P.W., Würzner, R., Oliveira, D.B.G., Lachmann, P.J., Peters, D.K., 1993. Complement-mediated adipocyte lysis by nephritic factor sera. J. Exp. Med. 177, 1827–1831.
Medof, M.E., Iida, K., Mold, C., Nussenzweig, V., 1982. Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. J. Exp. Med. 156 (December (6)), 1739–1754.
Müller-Eberhard, H.J., Nilsson, U., Aronsson, T., 1960. Isolation and characterization of two beta-1-glycoproteins of human serum. J. Exp. Med. 111, 201–215.
Nicol, P.A.E., Lachmann, P.J., 1973. The alternate pathway of complement activation: the role of C3 and its inactivator (KAF). Immunol 24, 259–275.
Nilsson, U.R., Mueller-Eberhard, H.J., 1965. Isolation of beta 1f-globulin from human serum and its characterization as the fifth component of complement. J. Exp. Med. 122 (2), 277–298.
Nilsson, B., Nilsson Ekdahl, K., 2012. The tick-over theory revisited: is C3 a contact-activated protein? Immunobiology 217, 1106–1110. https://doi.org/10.1016/j.imbio.2012.07.008.
Pangburn, M.K., Schreiber, R.D., Müller-Eberhard, H.J., 1981. Formation of the initial C3 convertase of the alternative complement pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. J. Exp. Med. 154, 856–867.
Pickering, M.C., Cook, H.T., 2008. Translational mini-review series on complement factor H: renal diseases associated with complement factor H: novel insights from humans and animals. Clin. Exp. Immunol. 151 (2), 210–230. https://doi.org/10.1111/j.1365-2249.2007.03574.x.
Pillemer, L., Blum, L., Lepow, L.H., Ross, O.A., Todd, E.W., Wardlaw, A.C., 1954. The properdin system and immunity: I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science 120, 279–285.
Rose, K.L., Paixao-Cavalcante, D., Fish, J., Manderson, A.P., Malik, T.H., Bygrave, A.E., Lin, T., Sacks, S.H., Walport, M.J., Cook, H.T., Botto, M., Pickering, M.C., 2008. Factor I is required for the development of membranoproliferative glomerulonephritis in factor H-deficient mice. J. Clin. Invest. 118 (February (2)), 608–618. https://doi.org/10.1172/JCI32525.
Roversi, P., Johnson, S., Caesar, J.J.E., McLean, F., Leath, K.J., Tsiftsoglou, S.A., Morgan, B.P., Harris, C.L., Sim, R.B., Lea, S.M., 2011. Structural basis for complement factor I control and its disease-associated sequence polymorphisms. PNAS 108 (August (31)), 12839–12844. https://doi.org/10.1073/pnas.1102167108.
Sadallah, S., Gudat, F., Laissue, J.A., Spath, P.J., Schifferli, J.A., 1999. Glomerulonephritis in a patient with complement factor I deficiency. Am. J. Kidney Dis. 33 (6), 1153–1157.
Scholl, H.P., Fleckenstein, M., Charbel Issa, P., Keilhauer, C., Holz, F.G., Weber, B.H., 2007. An update on the genetics of age-related macular degeneration. Mol. Vis. 13, 196–205.
Schreiber, R.D., Götze, O., Müller-Eberhard, H.J., 1976a. Alternative pathway of complement: demonstration and characterization of initiating factor and its properdin-independent function. J. Exp. Med. 144 (October (4)), 1062–1075.
Schreiber, R.D., Götze, O., Müller-Eberhard, H.J., 1976b. Nephritic factor: its structure and function and its relationship to initiating factor of the alternative pathway. Scand. J. Immunol. 5 (6-7), 705–713.
Scott, D.M., Amos, N., Sissons, J.G., Lachmann, P.J., Peters, D.K., 1978. The immunoglobulin nature of nephritic factor (NeF). Clin. Exp. Immunol. 32 (1), 12–24.
Scott, D.M., Amos, N., Bartolotti, S.R., 1981. The role of carbohydrate in the structure and function of nephritic factor. Clin. Exp. Immunol. 46 (1), 120–129.
Seya, T., Turner, J.R., Atkinson, J.P., 1986. Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b. J. Exp. Med. 163 (April (4)), 837–855.
Sim, R.B., Day, A.J., Moffatt, B.E., Fontaine, M., 1993. Complement factor I and cofactors in control of complement system convertase enzymes. Methods Enzymol. 223, 13–35.
Spitzer, R.E., Vallota, E.H., Forristal, J., Sudora, E., Stitzel, A., Davis, N.C., West, C.D., 1969. Serum C'3 lytic system in patients with glomerulonephritis. Science 164, 436–437.
Tamura, N., Nelson, R., 1967. Three naturally occurring inhibitors of components of complement in guinea pig and rabbit serum. J. Immunol. 99, 582–589.
Thompson, R.A., 1972. IgG3 levels in patients with chronic membranoproliferative glomerulonephritis. Br. Med. J. 975, 282–284.
Thompson, R.A., Lachmann, P.J., 1977. A second case of human C3b inhibitor (KAF) deficiency. Clin. Exp. Immunol. 27, 23–29.
Torisu, M., Sonozaki, H., Shiarishi, S., Nishioki, K., 1968. Purification of C3 inactivator from human serum. Nature 218, 1163–1164.
Whaley, K., Ruddy, S., 1976. Modulation of the alternative complement pathways by beta 1 H globulin. J. Exp. Med. 144 (November (5)), 1147–1163.
Yaseen, S., Demopulos, G., Dudler, T., Yabuki, M., Wood, C., Cummings, W., Tjoelker, L., Fujita, T., Sacks, S., Garred, P., Andrew, P., Sim, R., Lachmann, P., Wallis, R., Lynch, N., Schwaeble, W., 2017. The lectin pathway effector enzyme MASP-2 can activate native complement C3 in absence of C4 and/or C2. FASEB J. 31 (5), 2210–2219.
Ziegler, J.B., Alper, C.A., Rosen, F.S., Lachmann, P.J., Sherington, L., 1975. Restoration by purified C3b inactivator of complement-mediated function in vivo in a patient with C3b inactivator deficiency. J. Clin. Invest. 55, 668–672.