The Role of Complement in the Clearance of Cold Agglutinin-Sensitized Erythrocytes in Man

CHARLES J. JAFFE, JOHN P. ATKINSON, and MICHAEL M. FRANK

From the Section of Clinical Immunology, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

ABSTRACT To define the pathophysiologic mechanisms of cold agglutinin disease, we investigated a human model of this syndrome in normal volunteers and in patients with diminished levels of serum complement. Subjects received intravenous injections of autologous, chromated (^{51}Cr) erythrocytes which had been exposed in vitro to purified cold agglutinin preparations and to fresh autologous serum (as a source of complement). In vitro tests confirmed that such cells were coated with activated complement components (C3b), but not with immunoglobulin.

Studies of erythrocyte clearance and simultaneous organ scanning showed that erythrocytes sensitized with low levels of cold agglutinin primarily undergo reticuloendothelial sequestration by the liver rather than intravascular hemolysis. After the initial sequestration of C3b-coated erythrocytes, a fraction of the cells are released back into the circulation and survive normally thereafter. Both phenomena are dose dependent and closely follow the sequestration and release pattern observed with IgM isoagglutinin sensitization.

Experiments that used heated autologous serum as a source of C3 inactivator demonstrated that functionally intact C3b is required for hepatic sequestration. Erythrocytes coated with C3d were not cleared from the circulation. In vitro assays that used human macrophage monolayers suggested that the intrahepatic conversion of C3b to C3d is responsible for the release of sensitized erythrocytes back into the circulation.

The clearance of cold agglutinin-sensitized erythrocytes was compared to the clearance mediated by IgM isoagglutinin. We found that the rate of complement fixation by an IgM antibody proceeds so rapidly in vivo that the time for complement activation is not a factor in limiting the rate of hepatic sequestration. The major limiting factor appears to be the rate of liver blood flow.

Maximal in vitro coating of erythrocytes with C3d conferred protection from further cold agglutinin sensitization but not from IgM isoagglutinin-mediated clearance. This suggests a mechanism for the resistance to lysis observed in cells obtained from patients with the cold agglutinin syndrome and confirms the marked dependence of the site of C3 attachment on the site of membrane localization of the sensitizing antibody.

INTRODUCTION

Cold agglutinins characteristically are complement-fixing IgM autoantibodies with specificity for the Ii group of erythrocyte antigens (1, 2) and share the property of binding to erythrocytes in the cold and dissociating at 37°C. These antibodies are usually identified in two general clinical settings: most commonly, they appear transiently after infectious illnesses (often *Mycoplasma pneumonia*). Only rarely is this phenomenon associated with clinical hemolysis. Less frequently, cold agglutinins occur as monoclonal antibodies (often in the absence of underlying disease). This syndrome is usually associated with mild hemolytic anemia, although it is occasionally punctuated by severe hemolytic crises after ambient exposure to cold. In both of these clinical settings, hemolysis appears to be complement mediated. Careful analysis has suggested that complement activation by cold agglutinins is initiated at temperatures below 37°C, but can proceed at core body temperature (3).

Although many aspects of cold agglutinin function and the mechanism of cold agglutinin mediated hemoysis have been investigated, several phenomena have remained largely unexplained. It has been shown that

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erythrocytes obtained from patients with cold agglutinin disease survive longer in these patients than do cells obtained from normal donors (4, 5). Furthermore, in one patient transfused with erythrocytes exposed to cold agglutinin and complement in vitro, Lewis, et al. found an unusual clearance pattern with rapid erythrocyte clearance followed by a partial reappearance in the intravascular space (6). Moreover, despite the fact that the circulating erythrocytes of such patients are heavily coated with C3, most patients have a rather mild hemolytic process and their erythrocytes are *more* resistant to in vitro lysis by cold agglutinin and complement than are erythrocytes from normal individuals (5, 7).

By using both human and animal systems, our laboratory and others previously have investigated the role of IgM antibody and complement in the in vivo destruction of erythrocytes (8–10). In those studies the complement-dependent nature of the phenomenon was verified as was the predominant role of the liver in cell sequestration. It was shown that the cell bound C3b fragment is primarily responsible for IgM-mediated hepatic uptake and it was suggested that the sequestration and release pattern is a function of complement activation.

The experiments reported here examine the effects of complement activation on erythrocyte clearance in cold agglutinin disease and bring together in vitro and in vivo data to further delineate the mechanism of the cold agglutinin syndrome.

METHODS

Antibodies. The cold agglutinin antibody used throughout these studies was obtained from a patient with idiopathic cold agglutinin syndrome. The initial purification technique and subsequent characterization of the antibody (Step) have been previously reported (11). In addition, further purification was achieved by Sephadex G200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) fractionation and by sucrose density centrifugation (9). This material was free of hepatitis antigen as determined by radioimmunoassay (9), and was sterile, and pyrogen free. Functional activity of the cold agglutinin was assayed by a modification of the C1 fixation and transfer test (12), involving C1 fixation at 16°C rather than at 37°C. An estimation of the number of C3 molecules bound to erythrocytes/bound molecule of this cold agglutinin has been previously published by Rosse et al. using a modification of the method of Borsos and Leonard (11). Radioiodination of the cold agglutinin with I-125 was performed with a modification of the iodine monochloride method (13).

The purification and characterization of the IgM isoagglutinin has been reported in detail elsewhere (9). Rabbit anti-human C3b and C3d were kindly provided by Dr. Roger Spitzer. Functionally pure human C3 inactivator (C3 INA),[1] used in the in vitro studies, was obtained from the Cordis Laboratories, Miami, Fla.

Patients. Normal volunteers and patients with hereditary angioedema were hospitalized at the Clinical Center of the National Institutes of Health, Bethesda, Md. All had normal physical examinations and normal hematocrit, reticulocyte and leukocyte counts, negative direct and indirect Coombs tests, and had no detectable hepatitis B surface antigen (by radioimmunoassay). During the studies, patients' activities were not restricted and no medications were administered. Each volunteer participated in only one clearance study to minimize exposure to radioactivity.

Techniques for erythrocyte clearance and localization. Human erythrocytes were separated, chromium-51 radiolabeled, and sensitized with antibody using methods identical to those previously reported (9). In the initial studies, dilutions of cold agglutinin were added to an equal volume of an erythrocyte suspension (3.3×10^8 cells/ml) and incubated with mixing for 15 min at 16°C. Without washing, an equal volume of autologous fresh serum as a source of complement was added to the mixture for an additional 15 min at 16°C. A further incubation at 37°C for 15 min was then carried out to complete complement activation before twice washing the cells in warmed (37°C) saline to allow the cold agglutinin to dissociate. Such cells had C3b on their surface as shown by a positive immune adherence reaction and by direct agglutination by antibody to C3b (14). Approximately 10 ml of a suspension of 3.3×10^7 cells/ml was then administered intravenously into the subject. Survival and organ localization studies were performed as previously described (9). In a second group of studies, cells (3.3×10^8/ml) with membrane-bound C3b were incubated for 30 min at 37°C with an equal volume of the patient's own serum (heated at 56°C for 60 min and diluted in Veronal buffered saline). Incubation in such heated sera led to no further deposition of complement, but the C3 INA, which is stable at 56°C, converted the C3b to C3d, as verified by loss of immune adherence reactivity as well as by reactivity with monospecific antibody to C3d. The clearance and localization studies proceeded as above.

In vitro studies. Human alveolar macrophages were obtained from volunteers and patients by lavage of the lingual lobe through a fiber optic bronchoscope. Details of this technique as well as characterization, quantitation, and cellular analysis of the alveolar fluid have been published elsewhere (15). Alveolar macrophage monolayers were prepared by adding approximately 1.5×10^6 macrophages in Hank's buffered salt solution to 1×1 cm Lab Tek chambers (Lab Tek Products, Naperville, Ill.) and were incubated in 5% CO_2 at 37°C for 1 h before use. The resulting macrophage monolayers were overlaid with the erythrocyte preparations (see Results), and the percentage of rosettes was determined as previously reported (14).

Immune adherence assays were performed as previously described (16).

RESULTS

In vivo clearance in normal volunteers. Approximately 10 ml of autologous erythrocytes (3.3×10^7 cells/ml) coated in vitro with autologous serum complements components were injected intravenously into each volunteer. Cells prepared in the manner described above were not agglutinated and were Coombs negative with anti-immunoglobulin antibody. However, C3 could

[1] *Abbreviations used in this paper:* INA, inactivator; C3b, defined here as the opsonically active fragment of C3 responsible for erythrocyte clearance and the immune adherence reaction.

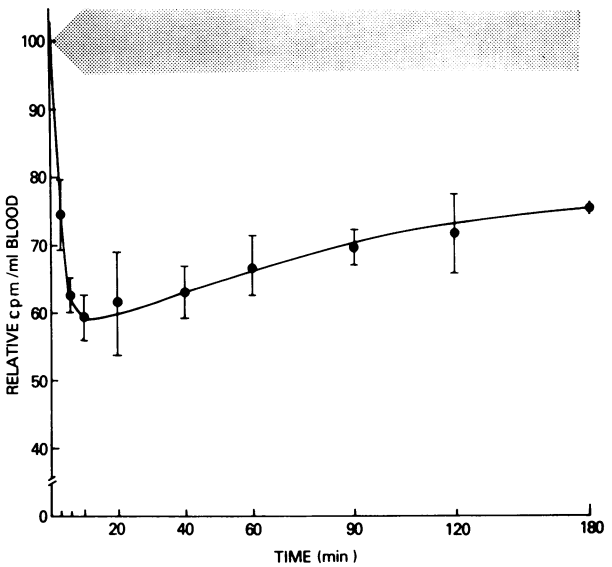

FIGURE 1 Clearance of cold agglutinin-sensitized erythrocytes in normal volunteers. Each point represents the mean (±1 SE) derived from studies of five normal volunteers, each of whose erythrocytes were sensitized with 150 Cl fixing sites per cell. The value at time 0 is derived from independent estimates of total blood volume. The shaded area represents the 95% confidence limits for the survival of chromated, unsensitized erythrocytes in five normal volunteers. Similar survival was observed when erythrocytes were incubated with cold agglutinin and then with heated (56°C, 30 min) rather than fresh serum.

be detected with appropriate Coombs reagents. The amount of C3 fixation with this preparation varied directly with the amounts of cold agglutinin. Previous studies with this antibody showed that 100 cell bound molecules of cold agglutinin fixed approximately 700 molecules of C3 (11). We also determined the number of bound cold agglutinin molecules by both the C1 fixation and transfer test and by the uptake of ^{125}I antibody (approximately one Cl fixing site per 10 molecules of bound cold agglutinin). This is in agreement with previously published results using this antibody (11).

Even at relatively low levels of complement coating, the radiolabeled erythrocytes were rapidly removed from the circulation during the first 10 min (Fig. 1). The time required for sequestration of one-half of the labeled erythrocytes was approximately 3½ min, with greater than 40% of the cells removed from the intravascular space during the first 6 min. There was a concomitant rise in radioactivity over the liver (Fig. 2). This rapid rate of removal suggests that hepatic blood flow is the limiting factor in sequestration. Shortly thereafter, and for at least the next 24 h, there was a progressive release of the sequestered erythrocytes into the circulation and a corresponding decrease in activity over the liver.

As the concentration of sensitizing antibody increased, and hence the amount of complement fixation increased, the degree of erythrocyte sequestration was altered directly. Thus, the greater the initial percentage of sequestration, the smaller the percentage of cells which eventually reappeared in the circulation (Fig. 3). With 75, 150, and 300 Cl sites per erythrocyte, 12, 39, and 67% of the injected cells were initially sequestered in the liver, and approximately 95, 79, and 50% respectively were returned to the circulation by 24 h. After 24 h, cells no longer were released into the intravascular space; the remaining cells survived with a half-life of 59 days. The kinetics of sequestration and release using cold agglutinin sensitization and complement coating in vitro were indistinguishable from results obtained when erythrocytes were sensitized in vitro with IgM isoagglutinin and subsequent complement fixation was allowed to proceed in vivo (Fig. 4). Thus, the time required for sensitized cells to interact with complement in vivo did not alter the kinetics.

To prepare erythrocytes coated with C3d, C3b erythrocytes prepared as described above were subsequently incubated at 37°C for 30 min with heated autologous serum (56°C for 60 min), which served as a source of C3 INA. Fig. 5 compares the sequestration and release pattern of erythrocytes coated with C3b and those coated with C3d, as verified by using monospecific

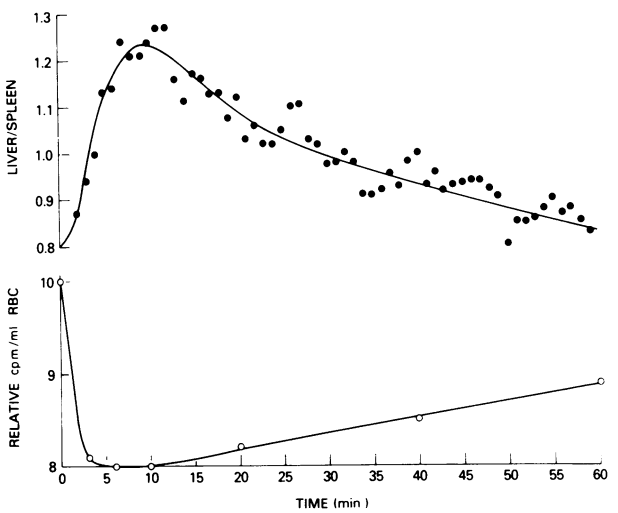

FIGURE 2 Organ localization of cold agglutinin-sensitized, chromated (^{51}Cr) erythrocytes after intravenous administration to one normal volunteer. The top portion of the graph represents the liver to spleen ratio of radioactivity detected by two NaI crystals over the respective organs. Each point (closed circles) was plotted from a calculated 3-point moving average of counts obtained over uninterrupted 1-min intervals. In the lower portion of the graph in the same volunteer study, the plotted values (open circles) reflect the intravascular clearance of erythrocytes sensitized with 100 Cl fixing sites per cell.

Coombs reagents. Whereas erythrocytes shown to be coated with C3b are rapidly sequestered before release, those cells coated with C3d survive normally.

In an effort to maximally coat erythrocytes with C3d, cell suspensions were sensitized initially with cold agglutinin and then twice cycled through the complement fixation procedure by repeated exposure to fresh serum first at 16°C then at 37°C followed by washing and incubation with a source of C3 INA. Further exposure of these cells to cold agglutinin and fresh serum did not lead to additional fixation of C3b as evidenced by the failure of the cells to mediate an immune adherence reaction or to exhibit accelerated erythrocyte clearance (Fig. 6). Agglutination of such cells by antibody to C3d confirmed its presence on the erythrocyte surface.

In a parallel experiment, erythrocytes maximally coated with C3d as above were subsequently exposed to IgM isoagglutinin instead of to cold agglutinin and fresh serum. Such cells were capable of further C3 fixation as evidenced by the typical sequestration and release pattern following intravenous administration (Fig. 6).

In vitro studies. Immune adherence studies that used microtiter techniques described previously were performed by using erythrocytes sensitized with 150 Cl fixing sites of cold agglutinin per cell. The results

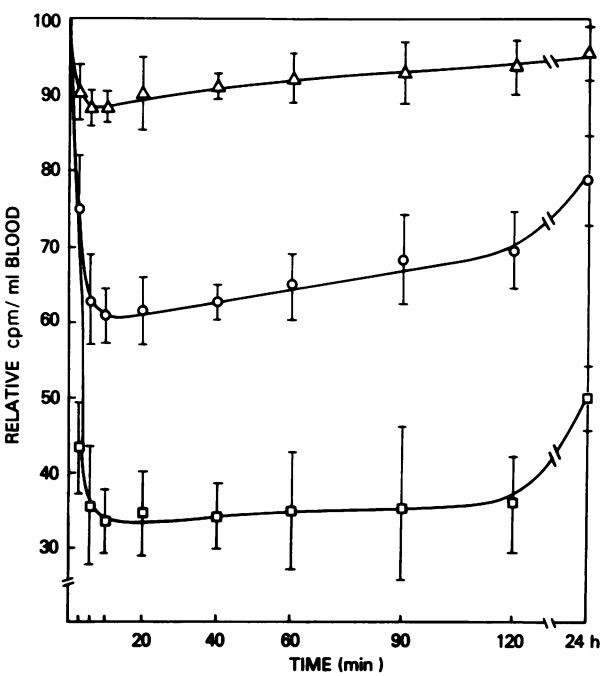

FIGURE 3 Dose dependence of cold agglutinin-sensitization on erythrocyte clearance in normal volunteers. Each point represents the mean (±1 SE) of blood samples obtained in multiple studies: (triangle) 75 Cl fixing sites/cell (two volunteers), (circle) 150 Cl fixing sites/cell (five volunteers), and (square) 300 Cl fixing sites per cell (two volunteers), respectively.

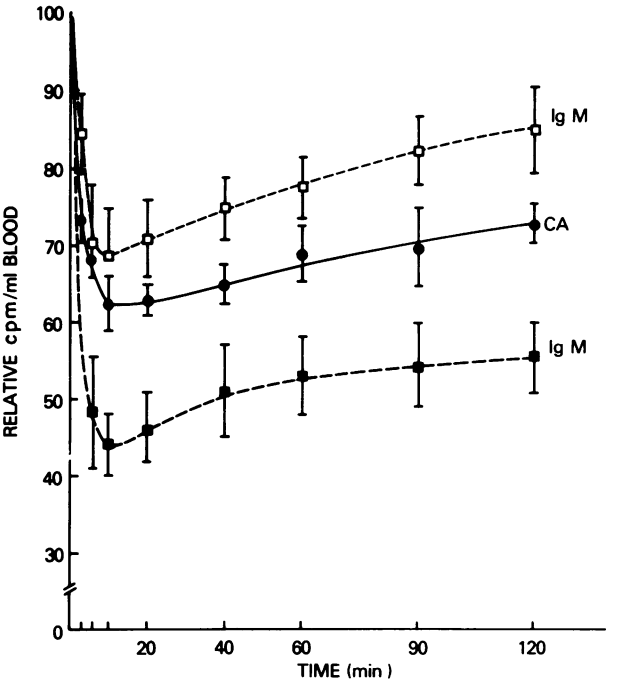

FIGURE 4 Comparison of the sequestration patterns produced by cold agglutinin-sensitization and by IgM isoagglutinin-sensitization. The solid line shows the pattern derived from values obtained by cold agglutinin-sensitization (150 Cl fixing sites pe cell in four volunteers). The shape of the curve is similar to those derived from isoagglutinin studies (broken line) in which erythrocyte sensitization was performed at two different levels: (open squares) 20 Cl fixing sites/cell in three volunteers, and (closed squares) 40 Cl fixing sites per cell in five volunteers. Each point represents the mean±1 SE.

(Table I) confirm two in vivo findings: (1) under the experimental conditions exposure to C3 INA effectively converts C3b to C3d and (2) repeated maximal complement fixation prevents subsequent complement fixation by cold agglutinin as evidenced by the loss of immune adherence reactivity, but does not prevent fixation by another IgM antibody directed at a different erythrocyte antigen.

In parallel studies, cells prepared by these techniques were overlayed on monolayers of human alveolar macrophages. The data (Table I) reaffirm the concept that erythrocytes coated with C3b but not with C3d are capable of rosetting with macrophages and that cells coated maximally with the C3d component do not form rosettes despite reexposure to the cold agglutinin and a fresh complement source.

In vivo clearance in complement deficient patients. Clearance studies in patients with hereditary angioedema were performed identically to those in normal volunteers (Table II). That is, erythrocytes sensitized with cold agglutinin were incubated with the patient's serum before readministration of the cells. In a patient whose level of C4 was approximately 5% of normal,

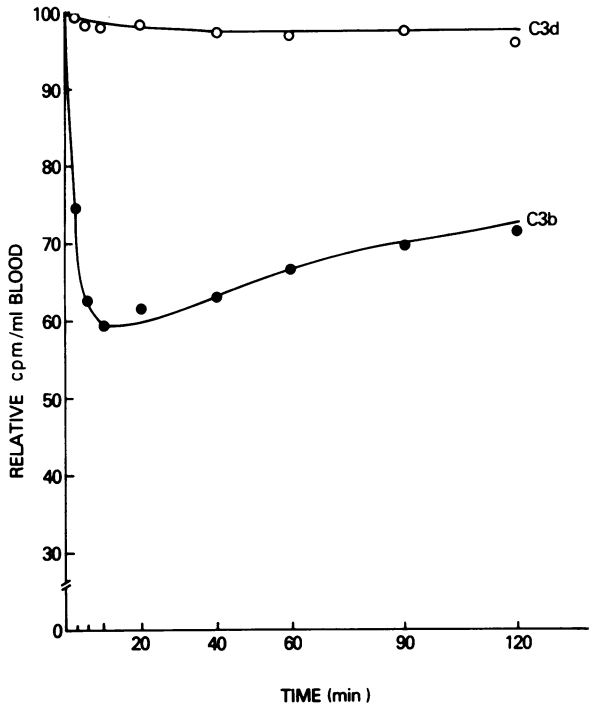

FIGURE 5 Clearance of cold agglutinin-sensitized erythrocytes after exposure to C3INA. (Solid circle) represents the mean of values obtained in five volunteers, each of whose erythrocytes were sensitized with 150 Cl fixing sites per cell, to yield C3b-coated erythrocytes. (Open circle) represents mean values derived from the study of two volunteers, whose cells were incubated with a source of C3INA, after sensitization with cold agglutinin (150 Cl fixing sites per cell) and complement. The presence of C3d on the erythrocyte surface was confirmed by agglutinination studies with monospecific anti-C3d.

immune adherence was detectable, C3b could be demonstrated with Coombs reagents, and a normal sequestration and release pattern was observed. However, in two patients in whom the C4 levels were less than 0.05% of normal and in whom no C2 could be detected, immune adherence and alveolar macrophage rosetting were not observed and no in vivo sequestration was noted.

DISCUSSION

In this study we have attempted to define the pathophysiology of cold agglutinin disease. Our first aim was to characterize the clinical syndrome in terms of its pathophysiologic manifestations. Secondly, we tried to define some of the fundamental mechanisms of in vivo complement action. In this regard the study of cold agglutinins is particularly useful because it is possible to dissociate the antibody from the erythrocyte surface after complement has been bound to the cell in vitro. By following the intravascular lifespan of such erythrocyte preparations, it further becomes possible to dissociate the in vivo actions of complement from those of the antibody.

The following points emerge from this series of studies. Autologous erythrocytes coated with low levels of cold agglutinin and allowed to interact with complement in vitro undergo clearance from the circulation rather than intravascular hemolysis. This supports the earlier findings of Evans et al. (4) and of Engelfriet et al. (5). It has been previously shown that when the quantity of sensitizing antibody is very large (17) or when the cold agglutinin is of unusual specificity (18), sufficient complement is activated at critical membrane sites to produce intravascular hemolysis. Nonetheless, cellular sequestration rather than intravascular hemolysis is the usual mechanism of complement-mediated cell damage in this disease.

Complement-coated erythrocytes free of sensitizing antibody are preferentially cleared by the liver. If in-

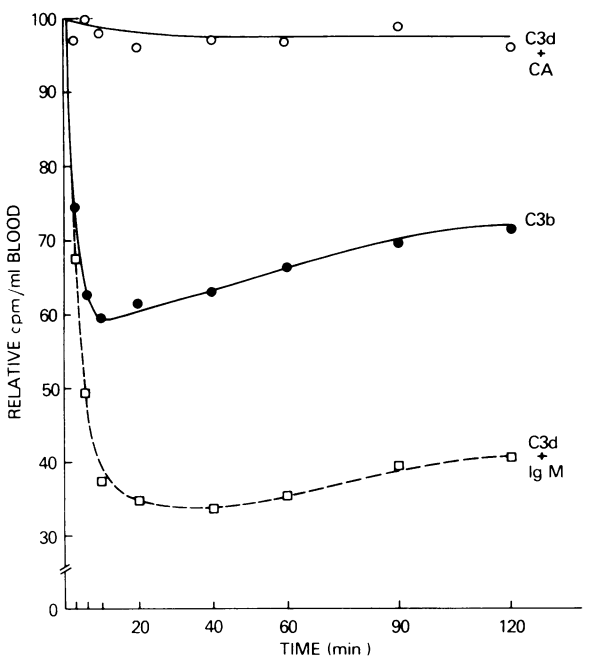

FIGURE 6 Clearance of erythrocytes maximally coated with C3d: effect of subsequent IgM isoagglutinin sensitization. (Open circle) represents values obtained from the study of two volunteers, whose erythrocytes were maximally coated with C3d by repeated exposure to cold agglutinin (CA), fresh serum, and then a source of C3 INA, before reexposure to cold agglutinin (used to achieve 150 Cl fixing sites per cell). (Open square) represents values obtained from the study of two volunteers, whose erythrocytes were maximally coated with C3d, before exposure to IgM isoagglutinin at 40 sites/cell. For comparison, the clearance of erythrocytes exposed to cold agglutinin (at 150 Cl fixing sites per cell) and then to complement is also shown (solid circle).

TABLE I

Correlation of In vitro Effects of Cell-Bound Complement with In vivo Erythrocyte Clearance

Erythrocyte preparation	C3 Fragment*	Immune adherence‡	Macrophage binding§	Intravascular clearance‖
EA	—	—	0	<5
EA + C	C3b	+ + +	80–99	50
EA + C + C3INA	C3d	—	4–7	5
EA + C + C3INA + IgM	C3d + C3b	+ + +	>90	>60

Abbreviations used: A, antibody (cold agglutinin); C, whole complement source; C3INA, C3 inactivator; E, erythrocyte; IgM, IgM isoagglutinin.

* Direct Coombs test using monospecific antisera.

‡ Visually scored in microtiter assay (16).

§ Percentage of macrophages which bind three or more erythrocytes.

‖ Percentage of administered erythrocytes cleared from the circulation by 20 min.

sufficient complement is activated at the erythrocyte surface to achieve hepatic sequestration, the cells undergo normal survival; at no dose of complement, could we demonstrate preferential splenic sequestration. The hepatic clearance of IgM isoagglutinin-coated cells was first established by the early studies of Jandl et al. (19) and Mollison (20). They suggested that entrapment by the liver was caused by extensive erythrocyte membrane damage. Evans and co-workers (4) were also able to demonstrate the hepatic sequestration of transfused cells in patients with cold agglutinin syndrome despite the apparent normal survival of a portion of the cells which remained in the circulation.

Our data suggest that complement (C3b) is essential for hepatic sequestration and confirm that the macrophage receptor for C3b is capable of binding these sensitized erythrocytes. Thus, the hepatic sequestration of cold agglutinin sensitized cells is more likely a function of erythrocyte binding at specific hepatic macrophage receptor sites for C3b rather than nonspecific membrane damage. In support of this, Brown and his colleagues (10) were able to show that cold agglutinins injected into rabbits produced rosetting on hepatic macrophages rather than causing cellular damage per se. Additional evidence substantiating the importance of complement in IgM mediated clearance in man comes from the findings that IgM isoagglutinin coated cells are not cleared in patients deficient in C4 and C2 (9), and that receptors for homologous IgM have not been identified on human alveolar macrophages (14), or on peripheral blood monocytes (14, 21).

A compatible, though not mutually exclusive hypothesis has been proposed, whereby C3b reduces erythrocyte deformability (22). Since enzymatic cleavage of C3b to C3d permits improved deformability, the authors argue that hepatic sequestration and release reflects a mechanical process within the hepatic microcirculation. This hypothesis does not explain the predilection of these complement-coated cells for the liver. Several authors have shown that minimally damaged erythro-

TABLE II

Effect of Cold Agglutinin Sensitization on Erythrocyte Clearance in Patients with Diminished Levels of Serum Complement

Patient	C4*	Immune adherence‡	Non-γ Coombs (C3b)§	Macrophage binding‖	Clearance
					%
1	5,200	+ + +	+ +	89	44
2	<100	0	0	<5	<5
3	<100	0	0	<5	<5
Controls**	110,000	+ + +	+ +	>90	50

* C4 titer by functional hemolytic assay (9).

‡ Visually scored by microtiter assay (16).

§ Direct Coombs test using monospecific antisera.

‖ Percentage of macrophages which bind three or more erythrocytes.

¶ Percentage of administered erythrocytes cleared from the circulation by 20 min.

** Data from five normal volunteers.

cytes are cleared by the spleen as are erythrocytes with defects in deformability (23). Nevertheless, splenic sequestration does not occur irrespective of the level of erythrocyte sensitization. Moreover, the concept of partially reversible deformability is not entirely consistent with the finding that cells from cold agglutinin patients survive longer in patients with the disease than do normal erythrocytes, unless the decrease in deformability is not functionally significant.

A possible in vitro correlate of the hepatic release phenomenon can be demonstrated by the conversion of the cell-bound C3b to C3d. This is achieved by exposing erythrocytes with functionally bound C3b to a source of the C3 INA (heated serum), thereby cleaving C3b to C3d. When administered intravenously, C3d coated erythrocytes are not cleared despite the presence of C3d receptors on macrophages and lymphocytes. We have previously suggested that such C3d is either not available to the macrophage receptors or is not in the proper biochemical or configurational form to mediate clearance (14). These data are in accord with that hypothesis. It has been reported that the circulating erythrocytes of patients with cold agglutinin syndrome are coated with C3d, but not C3b (5). Thus, the presence of C3d may preclude the sufficiently rapid fixation of further C3 (4, 5) or cold agglutinin (24), or simply reduce the amount of C3b below the threshold of receptor recognition. This too, would explain the observation of Evans et al. that previously sensitized erythrocytes survive longer than normal donor erythrocytes when injected into patients with active cold agglutinin syndrome (4).

Our human in vivo data demonstrate the phenomenon of hepatic sequestration and release. The findings are similar to those in a patient reported by Lewis, et al. (6) and those reported in a rabbit model by Brown et al. (10). The time course of cellular sequestration and release is identical whether antibody-free, C3-coated erythrocytes, or complement-free, IgM isoagglutinin-coated erythrocytes are administered intravenously. This suggests that in vivo complement fixation of the IgM coated cells proceeds so rapidly that it does not influence the rate of sequestration by the liver. Clearance appears to depend primarily upon the rate of hepatic blood flow. This is further supported by the finding that the rate of clearance of C3b-coated erythrocytes approximates that reported for other liver blood flow dependent colloid particulate materials (25, 26). Moreover, the shape of the sequestration and release curve does not reflect continued complement activation after the cells are injected (since the cells were washed free of antibody), but rather depends upon the interaction of cell bound complement and serum factors, presumably C3 INA. Recently, Logue et al. (17) established the importance of the rate and the amount of C3 fixation in the pathogenesis of the hemolytic state. Our data involving repeated complement exposure confirm and extend the premise relating the rate of complement fixation to the severity of the diseases. In fact, prolonged exposure to C3 and its inactivator, which is maximized in the in vitro model, confers "protection" on erythrocytes against further complement deposition. This protection by complement explains both the enhanced survival of in vivo coated cells when reinjected into patients with this syndrome (4, 5) as well as the observation of reduced susceptibility to in vitro lysis when compared with normal controls (5, 7). In fact, by using this particular cold agglutinin, enough C3d could be deposited so as to essentially prevent the further deposition of complement components. The finding that cold agglutinin antibodies are directed against only a portion of the erythrocyte antigen (27) may explain the variability of this protection phenomenon (11).

The data presented here on double-sensitization with a second IgM isoagglutinin also establish that the deposition of inactivated complement components at the I sites does not preclude subsequent fixation of complement at other more ubiquitous blood group antigen sites. The finding of a limited site for C3 binding by cold agglutinin is of importance in understanding the mechanisms of C3 activation. It has been shown that activation of C3 leads to the formation of an evanescent, fairly nonspecific binding site which allows the C3b fragment to attach to molecular species in the general area of activation (28). Furthermore, the active C3 fragment, unlike the active C5 cleavage product, cannot traverse large distances before binding (i.e., C3b unlike C5b does not transfer from sensitized to unsensitized sheep erythrocytes in a mixture of sensitized and unsensitized cells and complement) (29). These experiments in a human system confirm the limited extent of membrane fixation of C3b on individual cells and demonstrate the marked dependence of C3 fixation on the membrane localization of the sensitizing antibody.

Thus, these studies lead to a further understanding of the clinical manifestations of the cold agglutinin syndrome and to the pathophysiologic role of complement in the development of the disease. As suggested by Jandl et al. (19) complement activation at the erythrocyte surface is responsible for hepatic sequestration. Unlike previous hypotheses, however, we do not believe that complement-mediated damage per se produces this clearance phenomenon. Rather, we propose that sufficient complement activation at the erythrocyte surface yields C3b fragments capable of producing erythrocyte binding to specific hepatic macrophage receptors. In turn, if ingestion of the erythrocyte is not effected, C3 INA cleaves C3b to C3d resulting in cellular release and return to the circulating blood pool.

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