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Receptors on guinea-pig erythrocytes specific for cell-bound fourth component of human complement (C4)

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Summary. Guinea-pig erythrocytes have receptors for heterologous (human and rabbit) complement activated by the classical pathway on cell surfaces. This was shown in the present study by rosette-forming reactions of guinea-pig erythrocytes and human lymphocytes or sheep erythrocytes pre-treated with antibody and human R3 complement. The binding is temperature-dependent and is enhanced by treating the guinea-pig erythrocytes with neuraminidase. The receptors were shown to be specific for C4 by inhibition tests employing a range of anti-human complement antibodies (including anti-C1q, -C$\bar{1}$ inhibitor, -C4, -C2, -C3 and -C3b inactivator). Of these reagents, only anti-C4 inhibited the receptor activity, indicating that the guinea-pig erythrocyte C4-receptors differ from those on lymphocytes, monocytes, polymorphonuclear leucocytes and human erythrocytes which are reported to react with both C3b and C4b. In contrast to the strong affinity observed for heterologous C4, guinea-pig erythrocytes appear to react very weakly, if at all, with homologous C4.

Abbreviations: ALS, anti-human lymphocyte serum; ATS, anti-guinea-pig thymocyte serum; E_{GP}, untreated guinea-pig erythrocytes; Nase-E_{GP}, neuraminidase-treated guinea-pig erythrocytes; E_H, human erythrocytes; EAC_{GP} and EAC_H, sheep erythrocytes carrying IgM antibody and either guinea-pig or human complement.

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INTRODUCTION

An affinity of guinea-pig erythrocytes (E_{GP}) for activated complement was first shown as a rosette-forming reaction on rabbit lymphocytes (Wilson, Lachmann & Coombs, 1979a). Complement activation by both the classical and alternative pathways, occurs when these lymphocytes are isolated from defibrinated blood or are incubated in autologous serum, and results in the deposition of complement components on the cell surface (Wilson, Kanski & Coombs, 1978; Wilson *et al.*, 1979a). The adherence of E_{GP} to rabbit lymphocytes was shown to depend on complement activated by the classical pathway; no rosettes being formed by E_{GP} following alternative pathway activation. The binding of E_{GP} was enhanced by pre-treating them with neuraminidase, although papain and trypsin destroyed the reactivity. It was concluded that E_{GP} have receptors for at least one component of rabbit complement which is associated exclusively with the classical pathway, probably C1, C4 or C2. There are no other reports of similar receptors either on E_{GP} or on erythrocytes of any other species, although human erythrocytes have immune adherence

receptors for C3b which also react with C4b (Cooper, 1969).

Unfortunately, in our earlier study it was not possible to identify the component of rabbit complement reacting with E_{GP} receptors as no antisera were available for inhibition tests. It was, therefore, decided to change to a human system for which specific anti-complement reagents could be obtained, and the results of these experiments are the subject of the present report.

MATERIALS AND METHODS

Preparation of lymphoid cells

(a) *Human blood lymphocytes.* Blood was drawn by venepuncture from normal individuals and was either taken into 10 mM ethylenediaminetetra-acetate (EDTA) or defibrinated with a glass rod. Lymphocytes were isolated from EDTA–blood by first sedimenting erythrocytes with dextran (1 ml 6% Dextran 2000 per 10 ml blood) and then fractionating the leucocyte-rich suspension by Ficoll–Hypaque centrifugation according to the method of Böyum (1968). This yielded a mononuclear cell suspension containing lymphocytes and a variable number (up to 23%) of monocytes, assessed morphologically. A purer lymphocyte preparation (96–98%) was obtained from the defibrinated blood by treating with carbonyl iron and methyl cellulose at 37° (method described by Wilson & Coombs, 1971).

The lymphocytes were washed three times in either RPMI 1640 medium containing 5% heat-inactivated foetal calf serum (iFCS) or phosphate-buffered saline with 1% crystalline bovine serum albumin (PBS/BSA). They were finally resuspended to 4×10^6 cells per ml.

(b) *Human lymphoblastoid (Raji) cells.* These cells were taken from culture in RPMI 1640/10% iFCS, washed once in RPMI 1640/5% iFCS and twice in protein-free RPMI 1640 medium. They were adjusted to 1×10^6 cells per ml.

(c) *Guinea-pig lymph node lymphocytes.* Mesenteric or cervical lymph nodes were removed from normal guinea-pigs (Dunkin–Hartley or Frant strains) and the cells were isolated by teasing. Any clumps were removed by filtering, and the cells were washed three times and finally adjusted to 4×10^6 cells per ml in PBS/BSA.

Reagents

(a) *Antisera. Horse anti-human lymphocyte serum (ALS)* was kindly supplied by the Wellcome Research Laboratories, Beckenham, Kent. Before use, the serum was heat-inactivated and was extensively absorbed with human erythrocytes.

Sheep anti-guinea-pig thymocyte serum (ATS) was a gift from Professor A. K. Lascelles (Victoria, Australia). It was raised by injecting the sheep (V311) intravenously with well washed guinea-pig thymocytes. The serum was heat-inactivated and fully absorbed with guinea-pig erythrocytes before use.

Anti-human complement reagents. The antisera to human C1q (sheep serum 423), C3 (rabbit serum, R.I 14), C4 (sheep Z652 and Z892/D) and factor B (goat 122) were as described in Coombs, Wilson & Lachmann (1980). Sheep antiserum to $\overline{C1}$ inhibitor and rabbit antisera to C2 (R. 168), C5 (R. white), C7 (R. 202), C8 (R. 1511) and C3b-inactivator (R. 470) were raised to the purified proteins. These sera were absorbed either with human serum fractions, or (for C2 and C8) with genetically deficient sera. Anti-C6 (R. 5059) was made by injecting C6-deficient rabbits with normal rabbit C6. Sheep (A4) anti-C3 was a gift from Dr M. B. Pepys (London) and was produced by injecting purified C3 in Freund's complete adjuvant.

All sera were monospecific on testing by immunodiffusion against normal human serum. IgG-rich fractions were prepared either by DEAE–cellulose fractionation or by Rivanol–ammonium sulphate precipitation (Heide & Schwick, 1978).

Guinea-pig anti-guinea-pig C4 was raised in C4-deficient guinea-pigs, and was a gift from Dr D. Bitter-Suermann (Mainz, Germany). An IgG-rich fraction was produced by the Rivanol-ammonium sulphate procedure (Heide & Schwick, 1978).

(b) *Complement and C-yeast (R3) reagents.* Human and guinea-pig sera were separated from normal blood, and stored over liquid nitrogen. C-yeast reagents were prepared by treating human or guinea-pig serum with yeast *(S. cerevisiae)* for 45 min at 37°, according to the methods of Lachmann & Hobart (1978). These reagents are here given the designation R3, although human C-yeast preparation, unlike that of guinea-pig, retains substantial amounts of C3, and cells treated with it will have fixed C3 in addition to C1, C4 and C2.

(c) *Erythrocytes. Guinea-pig erythrocytes (E_{GP})* were taken from normal Dunkin–Hartley or Frant

strain guinea-pigs. Blood collected by cardiac puncture was mixed with EDTA (final concentration 10 mM), and the cells washed three times with PBS, taking care to remove buffy coat leucocytes. For rosette formation, they were resuspended to 1% in PBS.

Neuraminidase-treated guinea-pig erythrocytes (Nase-E_{GP}) were prepared by incubating 50 μl packed, washed E_{GP} in 50 units of neuraminidase [Behringwerke, *Vibrio comma* (cholerae), containing 500 neuraminidase units per ml] for 1 h at 37°. The cells were then washed three times and resuspended to 1% in either RPMI 1640/0·2% iFCS or PBS.

Human erythrocytes (E_H) of group (O) were taken into citrate or EDTA and washed three times with PBS. All buffy coat cells were removed, and the erythrocytes were used as a 1% suspension in PBS.

Ox erythrocytes coupled by chromic chloride to IgG anti-complement reagents. The method for chromic chloride-coupling of IgG to trypsin-treated ox erythrocytes was modified from that originally used by Coombs, Wilson, Eremin, Gurner, Haegert, Lawson, Bright & Munro (1977) and was described by Wilson, Prichard-Thomas & Coombs (1979b). The reagents coupled for the present investigations were sheep (A4) anti-human C3, sheep (Z892/D) anti-human C4 and guinea-pig anti-guinea-pig C4. As controls, ox erythrocytes were coupled to IgG fractions of normal sheep or normal guinea-pig sera. For rosetting, these cells were used as a 1% suspension in PBS, and were stored at 4° for up to 2 weeks.

Preparation of EAC. Sheep erythrocytes were first coated with rabbit IgM antibody and then incubated for 2 min at 37° in 10% human or guinea-pig complement (R3 preparations) diluted in complement fixation diluent (Oxoid). The reactions were stopped by adding Antrypol (Suramin B.P., Bayer (U.K.) Ltd) at 1 mg per ml, and the cells were washed three times before resuspending to 1% in PBS. Control sheep cells were treated with antibody and heat-inactivated complement.

Treatment of lymphocytes with antibody and complement

Human blood lymphocytes (4×10^6 per ml) were rotated for 30 min at room temperature in a dilution (1:50) of horse anti-human lymphocyte serum which had been pre-determined to give optimum fixation of complement on the lymphocyte cell surface. They were then washed three times in complement fixation diluent and incubated for 15 min at 37° in human R3 complement. They were finally washed three times in PBS/BSA and adjusted to 2×10^6 cells per ml for rosetting reactions.

Guinea-pig lymph node lymphocytes were similarly treated with sheep anti-guinea-pig thymocyte serum (dilutions between 1:10 and 1:50), and subsequently with 10% guinea-pig R3 complement.

Rosette-forming reactions with E_{GP}, Nase-E_{GP} and E_H

(a) *Adherence to lymphocytes carrying antibody-activated complement.* Aliquots, usually 25 μl, of human or guinea-pig lymphocytes (2×10^6 per ml) were mixed with equal volumes of 1% E_{GP}, Nase-E_{GP} or E_H in 5 × 50 mm plastic tubes (Sterilin, type RT 20) and centrifuged at 1700 r.p.m. (350 *g*) for 2 min. The cell pellets were incubated, undisturbed, in a 37° water-bath for 15 min before being resuspended and mounted with toluidine blue stain (approx. 0·05%) on silicone-treated microscope slides. At least 200 lymphocytes were examined from each preparation and a reaction with three or more erythrocytes was considered positive.

(b) *Adherence of E_{GP} and Nase-E_{GP} to EAC.* Relative size differences between the large guinea-pig erythrocytes and the much smaller sheep cells used for the EAC made it possible to carry out a rosette-forming reaction similar to that described above for complement-coated lymphocytes. In this case, rosettes of the EAC were formed around the guinea-pig erythrocytes by diluting the stock 1% suspensions of the E_{GP} and Nase-E_{GP} 1:50. Equal volumes of these suspensions and of 1% EAC carrying either human or guinea-pig complement were mixed, centrifuged and incubated at 37° as described for lymphocyte rosettes They were resuspended, mounted on siliconed slides and examined for rosette formation.

Detection of C3 and C4 on lymphocytes

Lymphocytes at a concentration of 2×10^6 per ml in PBS/BSA were centrifuged (350 *g*, 2 min) at 4° with equal volumes (25 μl) of 1% trypsin-treated ox erythrocytes carrying IgG anti-C3 or anti-C4 antibodies. The cell pellets were resuspended immediately, and were mounted with toluidine blue stain as described previously.

RESULTS

Reaction of guinea-pig erythrocytes with human complement on human lymphoid cells

Complement activated by the classical complement pathway on normal human peripheral blood lymphocytes

Preliminary investigations. In the earlier experiments on rabbit lymphocytes (Wilson *et al.*, 1979a), E_{GP} were shown to react with lymphocytes carrying complement activated by the classical pathway, which was probably initiated by auto-antibodies reacting with cell-surface antigens. Attempts were made, therefore, to reproduce this system by activating human complement by the classical pathway on the surface of human lymphocytes.

Normal human blood lymphocytes were treated with a horse anti-human lymphocyte serum (ALS), washed well, and then incubated in 10% human R3 serum as a source of complement. These cells were found to react strongly with E_{GP} and Nase-E_{GP} in temperature-dependent rosette-forming reactions. In one experiment, for example, only 54% of the lymphocytes reacted with Nase-E_{GP} at 4°, while at 25° and 37° there were 93% and 96% rosetted lymphocytes, respectively. In all further tests, the pelleted mixture of lymphocytes and guinea-pig erythrocytes were incubated at 37° for 15 min before resuspending.

The results of a representative experiment on human lymphocytes, pre-treated with ALS and human R3, are shown in Table 1. The majority of lymphocytes reacted strongly with anti-C3 and anti-C4, and, in addition to the rosetting reaction with E_{GP}, they showed strong immune adherence to E_H. As found in earlier experiments on rabbit lymphocytes (Wilson *et al.*, 1979a), neuraminidase treatment of the E_{GP} increased the percentage of reacting lymphocytes and produced stronger rosettes. Nase-E_{GP} were used, therefore, in all subsequent experiments.

Nase-E_{GP} did not react with lymphocytes treated either with ALS alone or with ALS and heat-inactivated human R3 (Table 1). The addition of EDTA to the human R3 also inhibited subsequent rosette formation with the guinea-pig erythrocytes, showing that complement activation is necessary to fix the reactive component(s) to the lymphocyte cell surface.

Inhibition of Nase-E_{GP} rosettes by anti-human complement. Human lymphocytes, pre-treated with ALS and human complement, were exposed to the IgG fractions of sheep or rabbit antisera raised against a series of human complement components. The lymphocytes were subsequently washed and tested for inhibition of Nase-E_{GP} rosette-formation (Table 2).

Table 1. Adherence of E_{GP}, Nase-E_{GP} and E_H to human peripheral blood lymphocytes pre-treated with ALS antibody and human complement (R3). Effect of heating the complement

Treatment of lymphocytes*	Percentage of lymphocytes reacting with				
	E_{GP}	Nase-E_{GP}	E_H	Anti-C3	Anti-C4
Untreated	0	0	0	2	3
ALS+R3	88	99	90	100	99
ALS+heated R3	0	0	0	4	3

* Lymphocytes were treated with anti-lymphocyte serum (ALS) for 30 min at RT. They were then washed and incubated in 10% human R3 for 15 min at 37°

Only anti-C4 antibodies had any effect on the adherence of Nase-E_{GP}, and in tests with two different sheep anti-human C4 reagents, both completely inhibited the reaction. This indicated that E_{GP} have an affinity for human activated C4, and was in agreement with the factor being associated with the classical complement pathway.

C4-deficient human serum as a source of complement. Lymphocytes were coated with ALS antibody, washed, and incubated in either C4-deficient human serum or human R3. Both cells were then examined for the adherence of Nase-E_{GP} (Table 3).

The results clearly showed that the guinea-pig erythrocytes do not react with lymphocytes treated with C4-deficient serum, and strengthens the evidence from the inhibition tests (above) that the adherence reaction is with C4.

It is interesting that lymphocytes in C4-deficient human serum were found to have fixed C3 (Table 3). This did not occur in the presence of EGTA/Mg^{2+}, and may have been due to the activation of the C1-bypass pathway (May & Frank, 1973).

Effect of complement dilution and length of incubation time with complement on the affinity of E_{GP}. Human R3 serum was diluted in complement fixation diluent to give doubling dilutions from 1/10 to 1/320. Following an incubation period of 15 min at 37°, the lymphocytes were washed and tested for adherence of E_{GP}, Nase-E_{GP} and E_H (Table 4).

Table 2. Test for inhibition of Nase-E_{GP} rosetting reaction by various anti-human complement reagents

Anti-complement reagent (IgG fractions, 1 mg per ml)	Percentage of lymphocytes* rosetting with Nase-E_{GP}
Sheep anti-C1q	94
Sheep anti-C$\bar{1}$ inhibitor	96
Rabbit anti-C2	89
Rabbit anti-C3	93
Rabbit anti-C3b inactivator	89
Sheep anti-C4	0
Rabbit anti-C5	99
Rabbit anti-C6	96
Rabbit anti-C7	96
Rabbit anti-C8	97
Goat anti-factor B	89
Normal sheep IgG	93
Normal rabbit IgG	97

* Human blood lymphocytes pre-treated with ALS and human R3. These cells were mixed with anti-complement antibody for 30 min at 4°, then centrifuged and washed twice before reacting with Nase-E_{GP}.

Table 3. Effect of substituting human C4-deficient serum for human R3 serum on the adherence of Nase-E_{GP} and E_H to human lymphocytes

Lymphocytes treated with*:	Nase-E_{GP}	Anti-C4	E_H	Anti-C3
ALS + human R3 serum	99	100	93	99
ALS + human C4-deficient serum	0	0	88	95
ALS alone	< 1	0	2	5

* Blood lymphocytes + ALS (1/50) 30 min, RT, washed three times. Incubated 37°, 15 min with 10% human R3 or C4-deficient serum, washed three times.

The difference in strength of reaction between untreated E_{GP} and Nase-E_{GP} was apparent at all dilutions of serum, and especially in the higher dilutions where the affinity reaction of Nase-E_{GP} remained strong. In fact, the adherence of Nase-E_{GP} appeared to be at least as sensitive, if not more sensitive, than the immune adherence of E_H for the detection of activated complement on the surface of these lymphocytes.

When lymphocytes, pre-coated with antibody, were incubated in 10% human R3 for varying periods of time (1 min to 4 h), rosette formation with Nase-E_{GP} remained maximal for at least 2 h, but was reduced after 4 h incubation (Table 5). Again, the reactivity of the neuraminidase-treated erythrocytes was much greater than that of untreated E_{GP} and remained higher than that of E_H when the reaction was declining after 4 h incubation. The rapid rise to maximum rosette formation with Nase-E_{GP} and E_{GP} suggests that the erythrocyte receptor is for some part of the C4b molecule which is gradually lost when the C4 is further broken down by C4-binding protein and C3b inactivator (Fujita, Gigli & Nussenzweig, 1978). This loss of affinity for the erythrocyte receptor was more apparent in reactions with untreated E_{GP} where the number of rosettes began to decline after 1 h incubation in R3 serum.

Complement activated by the alternative pathway on human lymphoblastoid cells

When human lymphoblastoid (Raji) cells are incubated in fresh, homologous serum, C3 is activated by

Table 4. Effect of diluting human R3 serum used to treat antibody-coated lymphocytes on subsequent reactions with E_{GP}, Nase-E_{GP} and E_H

	Percentage of lymphocytes reacting with		
Dilution of R3*	E_{GP}	Nase-E_{GP}	E_H
1/10	83	100	99
1/20	90	98	99
1/40	89	100	100
1/80	91	99	99
1/160	79	97	86
1/320	70w	87	83
No R3 added	<0	1	0

* Blood lymphocytes pre-treated with ALS, washed, then incubated in human R3 diluted in complement fixation diluent for 15 min, 37°.
w, weak reaction.

Table 5. Effect of varying the incubation time of antibody-coated human lymphocytes in complement (human R3) on reactions with E_{GP}, Nase-E_{GP} and E_H

	Percentage of lymphocytes reacting with		
Time of incubation in human R3* (min)	E_{GP}	Nase-E_{GP}	E_H
0 (not incubated)	0	1	0
1	97	92	100
2	93	96	100
5	94	98	100
15	94	100	98
60	80w	100	93
120	72w	100	93
240	47w	80w	60

* Blood lymphocytes pre-treated with ALS, washed, and incubated in 10% human R3 in complement fixation diluent. Reaction with complement stopped at appropriate times with Antrypol (1 mg per ml). Cells washed three times.
w, weak reaction.

the alternative pathway and C3b becomes attached to the cell-surface (McConnell & Lachmann, 1977).

Raji cells pre-treated with human complement gave a strong, positive reaction with anti-C3 coated ox erythrocytes, and a proportion of them formed immune adherence rosettes with E_H. They did not, however, react with Nase-E_{GP} (Table 6), thus confirming the earlier findings with complement-coated rabbit

Table 6. Alternative pathway complement activation on the surface of human lymphoblastoid (Raji) cells.* Testing for adherence of Nase-E_{GP} and E_H

	Percentage of Raji cells reacting with		
Raji cells + human C′	Nase-E_{GP}	E_H	Anti-human C3
Incubated 37°, 2 min	0	28	98
Incubated 37°, 15 min	0	45	100
Incubated 37°, 60 min	0	41	89

* 5×10^5 Raji cells suspended in 0·125 ml RPMI 1640 medium + 0·125 ml normal human serum. Reaction stopped with Antrypol (1 mg/ml).

lymphocytes (Wilson *et al.*, 1979a), that E_{GP} do not react with C3 or any other component of the alternative complement pathway.

Tests for reaction of E_{GP} with guinea-pig complement activated by the classical pathway on homologous lymphocytes

To find whether E_{GP} have affinity for homologous, activated C4, guinea-pig lymphocytes were treated with sheep anti-thymocyte serum (not T-specific), then washed well and incubated in guinea-pig complement (R3). After further washes, the lymphocytes were tested for reaction with E_{GP} and E_H. These erythrocytes did not form rosettes with untreated lymphocytes.

Despite testing a range of different concentrations of antibody and guinea-pig complement, no rosettes were formed with either untreated or Nase-treated E_{GP} (Table 7). Positive reactions were always obtained with E_H which reacted with between 80 and 100% of the treated lymphocytes. The presence of C4 on the cell membrane of these lymphocytes was confirmed (a) by strong reactions of up to 97% of cells with E_{ox} carrying guinea-pig antibodies to guinea-pig C4, and (b) by partial (65%) inhibition of E_H immune adherence rosettes when the lymphocytes were pre-treated with homologous anti-C4 antibodies.

Human and guinea-pig complement activated by the classical pathway on sheep erythrocytes

Sheep erythrocytes were coated with IgM rabbit antibody to produce EA complexes which were incubated at 37° for 2 min in either human R3 serum or guinea-

Table 7. Tests for reactivity of E_{GP} and Nase-E_{GP} with guinea-pig complement activated on guinea-pig lymphocytes*

Experiment	Percentage of lymphocytes reacting with			
	E_{GP}	Nase-E_{GP}	Anti-C4	E_H
1	0	< 1	97	96
2	1	0	95	85
3	0	< 1	NT	95
4	0	0	NT	79

* Guinea-pig lymphocytes treated with sheep ATS (dilutions between 1:10 and 1:50) for 30 min, at RT, then washed three times and incubated in 10% guinea-pig R3, diluted in complement fixation diluent, for 15 min 37°. Cells washed three times before rosetting.

NT, not tested.

pig R3 serum to produce EAC_H or EAC_{GP}. These cells were tested for adherence of E_{GP} and Nase-E_{GP}. Using a ratio of 50 EAC:1 E_{GP}, the mixture was centrifuged and the pellet incubated at 37° for 15 min prior to resuspending. Control tests were made with EA treated with heat-inactivated R3 sera, and were all negative.

Strong rosettes of EAC_H were seen around Nase-E_{GP}, and definite, but slightly weaker, reactions occurred with untreated E_{GP}. As in experiments on lymphocyte-bound C4, E_{GP} showed no affinity for guinea-pig C4 on EAC. There was, however, a very weak reaction between EAC_{GP} and Nase-E_{GP} in which approximately 25% of the guinea-pig cells reacted with between 1 and 6 EAC_{GP}.

These results reflect the earlier observations of strong reactions with human complement on lymphocytes, but also indicate that neuraminidase-treatment of E_{GP} may reveal low-affinity receptors for an homologous complement component, possibly C4.

DISCUSSION

Receptors for activated C4 have been demonstrated on guinea-pig erythrocytes. These receptors react strongly with human C4 when it is activated on the surface of carrier cells, such as lymphocytes or sheep erythrocytes and their binding is potentiated by neuraminidase-treatment of the E_{GP}. Similarly strong affinity reactions were originally described between Nase-E_{GP} and rabbit complement (Wilson *et al.*, 1979a), and this was presumably also a reaction with C4. In contrast, the receptors appear to react very weakly, if at all, with homologous guinea-pig C4.

The E_{GP} will adhere to antibody-coated cells which have been incubated in complement for only 1 min, indicating that C4b is the binding agent. If the complement-carrying cells are incubated for much longer periods (1–4 h) in complement, the reactivity with E_{GP} is diminished, probably as a result of the breakdown of C4b to C4d by the C3b-inactivator and C4-binding protein (Fujita *et al.*, 1978).

Concurrently with this work, a study has been made in this laboratory of the co-agglutination phenomenon (Bordet & Gengou, 1911) in which E_{GP} are aggregated by mixtures of whole serum (e.g. human, rabbit or bovine) and antisera raised against these sera. This reaction has now been shown to be specifically associated with C4/anti-C4 complexes (Coombs *et al.*, 1980). As with complement activated on lymphocytes, co-agglutination is not given by guinea-pig C4 when it is mixed with anti-C4 antibodies. The conclusions to be drawn from these results, and from those described in the present report, are that heterologous C4 will react with E_{GP} receptors either when fixed as C4b on cell surfaces or when aggregated by antibodies to C4.

Receptors for C4b have been shown on a number of other cells including human erythrocytes (Cooper, 1969), lymphocytes, monocytes and granulocytes (Bokisch & Sobel, 1974; Ross & Polley, 1974, 1975). Evidence from the work of these authors indicated that the C4b receptors on these cells are identical with those for C3b. This was demonstrated by co-capping of the lymphocyte receptors for C3b and C4b, and by inhibition tests with soluble C3 and C4.

The C4-receptors on guinea-pig erythrocytes are, therefore, different from those found, so far, on other cells, in that they react only with C4 and have no reactivity with C3. This specificity for C4 may make guinea-pig erythrocytes a useful marker for the detection of classical, as opposed to alternative, pathway complement activation.

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