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C3b receptor (CR1) expression on the polymorphonuclear leukocytes from patients with systemic lupus erythematosus

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SUMMARY

Polymorphonuclear leukocytes (PMN) C3b receptor (CR1) numbers have been measured in 14 normal individuals and 15 patients with SLE. The results in the normals showed that PMN possess three distinct pools of CR1. CR1 expression was lowest at 0°C (mean 86,000 ± s.e.m. 7,000), but increased when the cells were incubated at 37°C (125,00 ± 16,000) or when the cells were exposed to the chemotactic peptide N-formyl-methionyl-leucyl-penylalanine (FMLP, 10^{-5} mol 1) at 37°C (207,000 ± 21,000). The increased expression at 37°C was not dependent upon protein synthesis, an intact cytoskeleton or energy. Although the response to FMLP did not require *de novo* protein synthesis, increased CR1 expression was dependent upon an intact cytoskeleton and energy. All three PMN CR1 pools were reduced in patients with active SLE, but were normal in those in whom the disease was inactive. Serial studies performed on three SLE patients showed that PMN CR1 numbers were low during periods of disease activity and increased during remission. These data suggest that low PMN CR1 numbers in SLE are a consequence of the disease.

Keywords C3b receptor CR1 polymorphonuclear leukocyte SLE

INTRODUCTION

Human polymorphonuclear leukocytes (PMN) possess the complement receptors CR1, CR3 and CR4 (Ross & Lambris, 1982; Ross *et al.*, 1983; Frade *et al.*, 1985; Vik & Fearon, 1985). The primary function of PMN CR1 is to establish contact between soluble immune complexes and other opsonised particles before their ingestion. CR1 levels on PMN have been quantified using both polyclonal and monoclonal anti-CR1 antisera. Fearon (1980) reported that PMN expressed approximately 57,000 specific binding sites for CR1 using polyclonal antiserum, whilst Iida, Mornaghi & Nussenzweig (1982) reported 140,000 using the monoclonal antibody 57F and Hogg *et al.* (1984) reported 46,000 using the monoclonal antibody E11. These numbers are known to spontaneously increase when PMN are warmed to 37°C (Fearon & Collins, 1983) and a further 2- to 3-fold increase is seen when PMN are stimulated with the synthetic chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) (Berger *et al.*, 1984; Richerson *et al.*, 1985) or with low concentrations of the tumour-promoting phorbolester, phorbolmyristate acetate (PMA) (Changelian *et al.*, 1985). The increase in CR1 number which results from this stimulation is not accompanied by an increase in total cellular CR1 indicating that the additional receptors are being translocated from an internal pool (Changelian *et al.*, 1985). Further evidence for the existence of an internal pool of CR1 has come from the work of O'Shea *et al.* (1985) who showed that when PMN

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were disrupted and fractionated on sucrose gradients, CR1 could be immunoprecipitated from the plasma membrane and an enriched fraction which contained the Golgi apparatus. The ability of PMN *in vitro* to increase expression of CR1 in response to chemotactic factors may be of physiological relevance. Augmentation of CR1 *in vivo* would result in the increased efficiency with which PMN could bind to and eliminate opsonized immune complexes or bacteria at sites of inflammation.

Patients with systemic lupus erythematosus (SLE) have been demonstrated to have reduced erythrocyte CR1 numbers (Miyakawa *et al.*, 1981; Wilson *et al.*, 1982; Iida *et al.*, 1982; Walport *et al.*, 1985; Holme *et al.*, 1986), an abnormality which may be inherited or acquired as a result of the disease process. The specific mechanism whereby an acquired reduction in receptor numbers occurs has not been defined. In this study we have investigated CR1 levels on PMN from normal individuals and SLE patients. We have confirmed that there are three PMN pools of CR1, determined some of the structural and metabolic requirements for their expression and shown that numbers of CR1 are reduced in all three of these pools in patients with active SLE.

MATERIALS AND METHODS

Reagents. The following reagents were purchased from the sources shown: N-formyl-methionyl-leucyl-phenylalanine (FMLP) and cytochalasin B (both diluted in DMSO and stored at −70°C), colchicine, lummicolchicine, soybean trypsin inhibitor (SBTI), pepstatin A, leupeptin, cycloheximide, puromycin, dinitrophenol (DNP) bovine serum albumin (BSA), rotenone, antimycin A, dextran, trypan blue (Sigma Chemical Co. Ltd, Poole, Dorset, England), dibutylphthalate and dinonylphthalate (BDH Chemicals, Poole, Dorset, England), RPMI 1640 (Flow Laboratories, Irvine, Ayrshire, Scotland), Iodobeads (Pierce & Warriner, Chester, Cheshire).

Patients and controls. Fifteen patients with SLE (Tan *et al.*, 1982) and 14 healthy laboratory personnel were included in the study. Patients were examined for evidence of disease activity immediately before venous blood sampling.

Preparation of CR1 and its antiserum. CR1 was purified according to the method of Fearon (1979) and a polyclonal antiserum was prepared by the immunization of rabbits. The characteristics of this antiserum have been documented previously (Holme *et al.*, 1986). $F(ab')_2$ fragments of the anti-CR1 antiserum and non-immune rabbit IgG were prepared by pepsin digestion (Nisonoff, 1964). $F(ab')_2$ anti-CR1 was radiolabelled with ^{125}I by the chloramine T method (McConahey & Dixon, 1966).

Preparation of cells. Sixty millilitres of blood was collected in heparinized containers. Ten millilitres of this was centrifuged and the plasma and buffy coat were removed. The red cell pellet was then washed three times in RPMI 1640 containing 1% (w/v) BSA (RPMI/BSA) and resuspended to $2{\cdot}5 \times 10^8$/ml in RPMI/BSA. The remaining 50 ml was layered on to Ficoll Hypaque (SG 1·077) and centifuged at 700 *g* for 30 min at room temperature. The mononuclear layer was then removed and the remaining blood was mixed with an equal volume of autologous plasma and allowed to sediment at 37°C in the presence of 6% (w/v) dextran. The PMN-rich fraction was further purified by hypotonic lysis and the purified PMN suspension was standardized to 5×10^6/ml in RPMI/BSA containing EDTA (20 mM) (RPMI/BSA/EDTA). Using the trypan blue exclusion test, the viability of PMN in each preparation was shown to be greater than 95%.

Treatment of PMN with drugs. PMN (5×10^6/ml) in RPMI/BSA/EDTA were incubated on ice for 30 min. Colchicine (10^{-5} mol/l), lummicolchicine (10^{-5} mol/l), cytochalasin B (10^{-5} mol/l), puromycin (40 μg/l $\times 10^6$ cells), cycloheximide (200 μg/l $\times 10^6$ cells), DNP (3×10^{-5} mol/l), rotenone (10^{-4} mol/l), and antimycin A (0·01% w/v) were added and the incubation on ice was continued for a further 10 min. FMLP (10^{-5} mol/l) was then added and the cells were warmed to 37°C for 30 min in a shaking water bath. Cells were centrifuged at 1500 *g* for 3 min at 4°C and resuspended in RPMI/BSA/EDTA containing 0·1% SBTI, 2·5 μg/ml pepstatin A and 2·5 μg/ml leupeptin (RPMI/BSA/EDTA/inhib).

Determination of erythrocyte and PMN CR1 number. Erythrocyte CR1 number was determined by the method described in detail by Holme *et al.* (1986). PMN CR1 number was determined by a

modification of this method. Briefly, PMN were suspended in RPMI/BSA/EDTA/inhib at 5×10^6/ml. Aliquots (200 μl) of the suspension were transferred to a series of eight microcap tubes. Unlabelled non-immune rabbit $F(ab')_2$ fragments (12·5 μg/tube) were added to tubes 1 to 4 and unlabelled anti-CR1 $F(ab')_2$ fragments (12·5 μg/tube) were added to tubes 5 to 8. These tubes were mixed end over end at 4°C for 30 min after which incremental quantities of ^{125}I anti-CR1 $F(ab')_2$ (1 μg, 2μg, 3 μg, 4 μg) were added to each set of four tubes. After a further incubation of 60 min at 4°C the samples were layered onto 300 ml of dibutylphthalate/dinonylphthalate in microfuge tubes and centrifuged at 10,000 *g* for 2 min. The amount of cell bound radioactivity was then determined and the number of antigenic sites per cell was obtained from a specific binding curve using Scatchard plot analysis (Scatchard, 1949).

Determination of PMN CR1 number using the monoclonal antibody E11. IgG antibody E11 (a gift from Dr Nancy Hogg) was isolated from mouse ascites fluid (Hogg *et al.*, 1984) and radiolabelled using Iodobeads.

Triplicate aliquots (200 μl) of PMN (5×10^6/ml) in RPMI/BSA/EDTA/inhib were incubated with incremental quantities of ^{125}I-E11 (0·1 μg to 2 μg) for 1 h at 4°C. Nonspecific binding of E11 was assessed by firstly incubating triplicate aliquots of PMN 5×10^6/ml with a 10-fold molar excess of unlabelled E11 for 30 min at 4°C before addition of ^{125}I-E11. The samples were treated in the same way as described for the polyclonal antiserum and the number of E11 antigenic sites determined by Scatchard plot analysis. Determination of CR1 number by E11 was used in more recent experiments (those shown in Figs 2 and 4B) as supplies of $F(ab')_2$ anti-CR1 were limited. CR1 numbers were determined on PMN from three individuals using both the polyclonal antibody and E11. In all three cases the number of sites detected with the polyclonal reagent was 2·5 times greater than the number measured with E11. In order to bring these data into line with the majority of the data which had been obtained with the polyclonal $F(ab')_2$ anti-CR1 we multiplied the number of CR1 sites determined with E11 by 2·5.

Statistical analysis. As CR1 numbers in normal and in patient groups were distributed in a non-parametric fashion, data were analysed using a Mann-Whitney *U* test.

RESULTS

CR1 numbers on normal PMN. PMN from the 14 normal individuals expressed 88,000 (mean) $\pm$ 7,000 (standard error of mean) CR1 antigenic sites per cell at 0°C, 125,000 ($\pm$16,000) at 37°C and 207,000 ($\pm$21,000) at 37°C in the presence of FMLP (10^{-5} mol 1) (Fig. 1). The difference in CR1 numbers at 0°C and at 37°C was significantly different ($P < 0{\cdot}05$). The number of CR1 expressed in the presence of FMLP was significantly greater than that expressed at 37°C ($P < 0{\cdot}05$).

Effect of protein synthesis inhibitors on expression of CR1. PMN were stimulated with FMLP in the presence of cycloheximide and puromycin. Neither of these two protein synthesis inhibitors were found to have any effect on the stimulation of CR1 expression with FMLP (Fig. 2) ($P < 0{\cdot}05$ in both cases). As protein synthesis inhibitors did not reduce CR1 expression in the presence of FMLP no studies were performed on the effect of these drugs on CR1 expression at 37°C.

Effect of inhibitors of cytoskeleton assembly on stimulation of CR1 expression. Cytochalasin B and colchicine did not affect the increase in CR1 expression which was seen upon warming PMN to 37°C (data not shown). However, both of these drugs abrogated the increased expression of CR1 seen in the presence of FMLP (Fig. 3). Lummicolchicine, a functional analogue of colchicine which does not affect microtubule structure (Wilson & Friedkin 1967), had no effect on FMLP-stimulated expression of CR1 (data not shown). These results indicate that microtubule and microfilament assembly are important for the mobilization of CR1 in response to FMLP. The observation that these drugs had no effect on the increased CR1 expression at 37°C shows that the cytoskeleton is not required. The specificity of the colchicine response is indicated by the observation that lummicolchine did not prevent the FMLP induced increase in CR1 numbers.

Suppression of FMLP-induced increase of CR1 with energy inhibitors. Three energy inhibitors, DNP, rotenone and antimycin A were found to inhibit the FMLP-induced expression of CR1 (Fig.

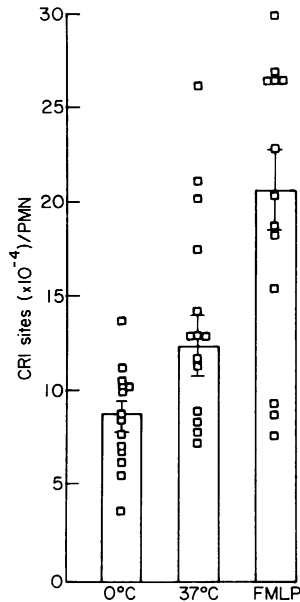

Fig. 1. Distribution of CR1 number (determined by polyclonal F(ab′)$_2$ anti-CR1) on PMN from 14 normal individuals after incubation at 0°C, 37°C or in the presence of FMLP (10^{-5} mol/l). The top of each bar represents the mean and the horizontal line represents the standard error of the mean (s.e.m.).

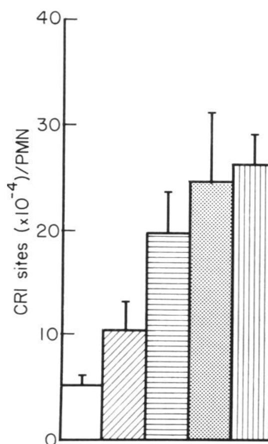

Fig. 2. Distribution of CR1 number at 0°C (□), at 37°C (▨), at 37°C in the presence of FMLP (10^{-5} ml/l, ▤), and at 37°C in the presence of FMLP plus puromycin (40 μg/l × 10^6 cells; ▩), and at 37°C in the presence of FMLP plus puromycin (40 μg/l × 10^6 cells; ▩), or cycloheximide (200 μg/l × 10^6 cells; ■). Each bar represents the mean ± s.e.m. of three experiments). CR1 numbers were determined with monoclonal antibody E11, and multiplied by 2·5 to bring the results into line with those obtained using the polyclonal anti CR1 antibody.

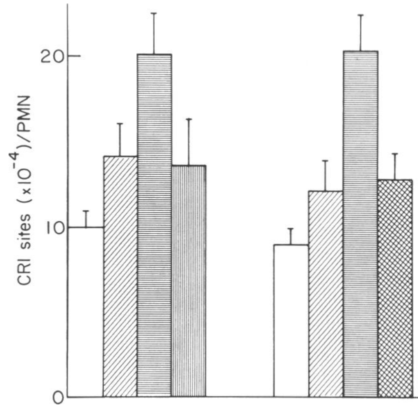

Fig. 3. Distribution of CR1 number (determined by polyclonal $F(ab')_2$ anti-CR1) at 0°C (□), at 37°C, (▨) at 37°C in the presence of FMLP (10^{-5} mol/l, ▤) and at 37°C in the presence of FMLP plus colchicine (10^{-5} mol/l ▥), or cytochalasin B (10^{-5} mol/l, ▩). Each bar represents the mean ± s.e.m. of nine experiments.

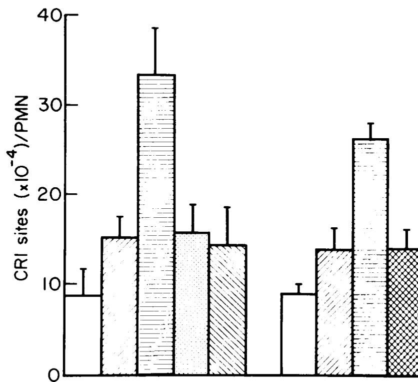

Fig. 4. Distribution of CR1 number at 0°C (□), at 37°C (▨), at 37°C in the presence of FMLP (10^{-5} mol/l, ▤), and at 37°C in the presence of FMLP plus DNP (3×10^{-5} mol/l, ▩), or rotenone (10^{-4} mol/l, ▧ or antimycin A (0·01% w/v; ▨). CR1 numbers for DNP treated PMN were determined by polyclonal $F(ab')_2$ anti-CR1. CR1 numbers for rotenone and antimycin A-treated PMN were determined using the monoclonal antibody E11, and multiplied by 2·5 to bring them into line with those obtained using the polyclonal antibody. Each bar represents the mean ± s.e.m. of five experiments.

4) ($P < 0·05$ for all inhibitors). These inhibitors did not effect the increased expression of CR1 at 37°C (data not shown).

CR1 numbers on PMN and erythrocytes from patients with SLE. Seven of the 15 SLE patients had active disease and eight had inactive disease at the time of study. The mean erythrocyte CR1 number from the seven patients with active disease was significantly lower (390 ± 308 sites/cell) than the eight patients in inactive disease (2770 ± 807 sites/cell) $P < 0·001$ (data not shown).

The number of CR1 sites expressed at 0°C and at 37°C in the absence and presence of FMLP in patients with active SLE were 15,000 ± 8,000, 68,000 ± 22,000 and 69,000 ± 23,000 respectively. These numbers were significantly lower than in those with inactive disease 125,000 ± 37,000, 166,000 ± 63,000 and 209,000 ± 57,000 respectively ($P < 0·05$ in all cases) (Fig. 5). These data also show that the PMN from SLE patients with active disease do not show increased membrane expression of CR1 in response to FMLP exposure.

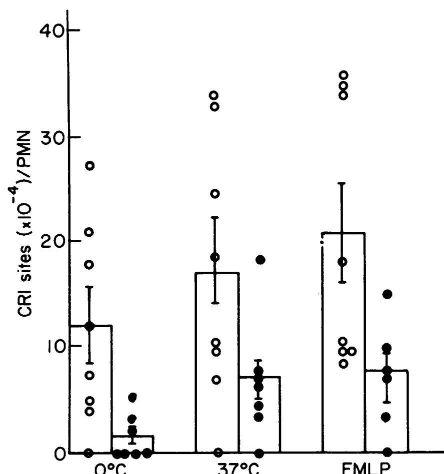

Fig. 5. Distribution of CR1 number (determined by polyclonal $F(ab')_2$ anti-CR1) on PMN from seven SLE patients with active disease (●) and eight with inactive disease (○) after incubation at 0°C, 37°C, or in the presence of FMLP (10^{-5} mol/l). Assays on FMLP-treated PMN were performed on only six patients with active SLE.

Table 1. Changes in PMN and erythrocyte CR1 numbers with disease activity in three patients with SLE

	CR1 sites/cell			
	PMN*		E	
Patient	Active	Inactive	Active	Inactive
1	41847	53146	0	440
2	0	257927	361	2859
3	70048	185669	2876	9526

* PMN CR1 were measured at 37°C.

Serial studies. CR1 numbers on erythrocytes and PMN were measured in three patients during periods of disease activity and also during remission. In the active state CR1 numbers were low on both erythrocytes and PMN whereas they increased on both cell types when the disease became inactive (Table 1).

Correlation between CR1 sites on erythrocytes and PMN. In normal individuals there was a significant correlation ($r=0{\cdot}77$) between the number of CR1 expressed on PMN at room temperature and the number of CR1 sites per erythrocyte ($P<0{\cdot}05$). Similar results were found in SLE patients where the number of PMN CR1 expressed at 0°C, at 37°C and in the presence of FMLP correlated significantly with the number of CR1 sites per erythrocyte ($r=0{\cdot}57$, 0·57 and 0·59 respectively; $P<0{\cdot}05$ in all cases). Correlations were also found between PMN CR1 expression at 0°C and at 37°C ($r=0{\cdot}94$; $P<0{\cdot}001$) between CR1 expression at 0°C and in the presence of FMLP ($r=0{\cdot}94$; $P<0{\cdot}001$) and between CR1 expression at 37°C and in the presence of FMLP ($r=0{\cdot}96$, $P<0{\cdot}001$).

DISCUSSION

SLE is a disease which is associated with a number of defects in the immune system, one of which is the presence of increased quantities of circulating antigen-antibody complexes (IC) (Tan *et al.*, 1982). Patients with SLE have been shown to have a deficiency in erythrocyte CR1 numbers (Miyakawa *et al.*, 1981; Iida *et al.*, 1982; Wilson *et al.*, 1982; Holme *et al.*, 1986). One of the major roles proposed for erythrocyte CR1 is the transport of soluble IC from the circulation to the phagocytic cells of the liver and spleen (Siegel, Liv & Gleicher, 1981; Cornacoff, 1983; Sherwood & Virella, 1986). Low numbers of erythrocyte CR1 in patients with active SLE may impair the capacity of erythrocytes to perform this function. Few studies have been performed on leukocytes from patients with SLE to determine if low CR1 numbers are a general feature of the disease. Wilson *et al.* (1986) have reported that the total number of CR1 on the neutrophils and B lymphocytes of SLE patients were reduced by 41% and 39% respectively but no relationship between CR1 numbers and serum levels of C3, C4 or immune complexes was found. No mention of the relationship between reduced PMN CR1 numbers and clinical evidence of disease activity was documented. Hurst, Nuki & Wallington (1984) reported a significant reduction in the rate of complement receptor mediated phagocytosis by blood monocytes in patients with SLE. This reduction was most evident in patients with active disease.

In agreement with others (Fearon & Collins, 1983; Berger *et al.*, 1984; Richerson *et al.*, 1985) we have found that CR1 expression can be increased by warming PMN to 37°C and further increased by stimulation with FMLP (10^{-5} mol/l) (Fig. 1). This increased expression was not the result of *de novo* CR1 synthesis as it was not inhibited by the presence of puromycin or cycloheximide (Fig. 2).

Our results with energy inhibitors suggest that CR1 which are brought to the surface upon warming to 37°C come from a different source than do CR1 which are expressed after stimulation with FMLP. DNP is a drug which uncouples the electron transport chain from the production of energy by preventing the phosphorylation of ADP to ATP. Rotenone and antimycin A both inhibit energy production by blocking electron transport flow between reduced nicotinamide adenine dinucleotide (NADH) and cytochrome b and between cytochrome b and cytochrome c respectively. In the presence of these drugs expression of CR1 at 37°C was normal. However, the increased expression following exposure to FMLP did not occur (Fig. 4). This finding implies that the translocation of CR1 from an internal pool is an energy dependent process while CR1 brought to the surface in response to warming to 37°C does not require energy. These results in conjunction with the results from experiments using colchicine and cytochalasin B (Fig. 5) indicate that there are three distinct pools of PMN CR1. One pool is expressed on the membrane at 0°C. A second is expressed at 37°C and does not require energy or an intact cytoskeleton for its expression, while a third pool which is expressed in the presence of chemotactic agents depends upon energy and an intact cytoskeleton for translocation to the membrane. Whether differentiation between pools one and two is of any value is questionable as PMN *in vivo* circulate at 37°C.

The only published study on PMN CR1 numbers in SLE patients measured receptor numbers by radioimmunoassay (Wilson *et al.*, 1986). These authors showed that the total number of PMN CR1 was reduced in these patients. Our findings confirm these observations and also show that all three pools of PMN CR1 were reduced in SLE patients with active disease (Fig. 5). The finding that PMN CR1 numbers rose during remission (Table 1) suggests that this abnormality is an acquired defect. Previous studies have shown that erythrocyte CR1 numbers are reduced during periods of increased disease activity and tend to return to normal during remission (Ross *et al.*, 1985; Holme *et al.*, 1986). As with the reduced erythorocyte CR1 numbers in patients with active SLE, the cause of reduced PMN CR1 is unknown and must merit further investigation. The consequences of reduced PMN CR1 in SLE patients is presently unknown. However PMN CR1 are thought to be important in the phagocytosis of opsonized bacteria, and in the internalisation and degradation of IC (Ehlenberger & Nussenzweig, 1977; Changelian *et al.*, 1985). Thus reduced CR1 expression by PMN could predispose to bacterial infection, a well known complication of SLE (Steinberg 1985), and also contribute to the severity and duration of IC-mediated tissue injury.

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REFERENCES

BERGER, M., O'SHEA, J., CROSS, A.S., FOLKS, T.M., CHUSED, T.M., BROWN, E.J. & FRANK, M.M. (1984) Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J. clin. Invest.* **74,** 1566.

CHANGELIAN, P.S., JACK, R.M., COLLINS, L.A. & FEARON, D.T. (1985) PMA induces the ligand independant internalisation of CR1 on human neutrophils. *J. Immunol.* **134,** 1851.

CORNACOFF, J.B., HERBERT, L.A., SMEAD, W.L. VAN AMAN, M.E., BIRMINGHAM, D.J. & WAXMAN, F.J. (1983) Primate erythrocyte-immune complex-clearing mechanism. *J. clin. Invest.* **7,** 236.

EHLENBERGER, A.G. & NUSSENZWEIG, V. (1977) The role of membrane receptors for C3b and C3d in phagocytosis. *J. exp. Med.* **145,** 357.

FEARON, D.T. (1979) Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *Proc. natn Acad. Sci. USA.* **76,** 5867.

FEARON, D.T. (1980) Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte and monocyte. *J. exp. Med.* **152,** 20.

FEARON, D.T. & COLLINS, L.A. (1983) Increased expression of C3b receptors on polymorphonuclear leukocytes induced by chemotactic factors and by purification procedures. *J. Immunol.* **130,** 370.

FRADE, R., MYONES, B.L., BAREL, M., KIRKORIAN, L., CHARRIAUT, C. & ROSS, G.D. (1985) GP140, a C3b binding membrane component of lymphocytes is the B cell C3dg/C3dR (CR2) and is distinct from the neutrophil C3dg receptor CR4. *Eur. J. Immunol.* **15,** 1192.

HOGG, N., ROSS, G.D., JONES, D.B., SLUSARENKO, M., WALPORT, M.J. & LACHMANN, P.J. (1984) Identification of an anti-monocyte monoclonal antibody that is specific for the membrane complement receptor type one (CR1). *Eur. J. Immunol.* **14,** 236.

HOLME, E., FYFE, A., ZOMA, A., VEITCH, J., HUNTER. J. & WHALEY, K. (1986) Decreased C3b receptors (CR1) on erythrocytes from patients with systemic lupus erythematosus. *Clin. exp. Immunol.* **63,** 41.

HURST, N.P., NUKI, G. & WALLINGTON, T. (1984) Evidence for intrinsic cellular defects of complement receptor-mediated monocyte phagocytosis in patients with systemic lupus erythematosus (SLE). *Clin. exp. Immunol.* **55,** 303.

IIDA, K., MORNAGHI, R. & NUSSENZWEIG, V. (1982) Complement receptor (CR1) deficiency in erythrocytes from patients with systemic lupus erythematosus. *J. exp. Med.* **155,** 1427.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunological studies. *Int. Arch. Allergy appl. Immunol.* **29,** 185.

MIYAKAWA, T., YAMADA, A., KOSAKA, K., TSUDA, F., KOSUGI, E. & MAYUMI, M. (1981) Defective immune adherence (C3b) receptor on erythrocytes from patients with systemic lupus erythematosus. *Lancet* **ii,** 493.

NISONOFF, A. (1964) Enzymatic digestion of rabbit gammaglobulin and antibody and chromatography of the digestion products. *Methods med. Res.* **10,** 134.

O'SHEA, J.J., BROWN, E.J., GAITHER, T.A., TAKAHASHI, T. & FRANK, M.M. (1985) Tumor promoting phorbol esters induce rapid internalisation of the C3b receptor via a cytoskeleton-dependent mechanism. *J. Immunol.* **135,** 1325.

RICHERSON, H.B., WALSH, G.M., WALPORT, M.J., MOQBEL, R. & KAY, A.B. (1985) Enhancement of human complement receptors: a comparison of the rosette technique with the uptake of radio-labelled anti-CR1 monoclonal antibody. *Clin. exp. Immunol.* **62,** 442.

ROSS, G.D. & LAMBRIS, J.D. (1982) Identification of a C3bi-specific membrane complement receptor that is expressed on lymphocytes, monocytes, neutrophils and erythrocytes. *J. exp. Med.* **155,** 96.

ROSS, G.D., NEWMAN, S.L., LAMBRIS, J.D., DEVERY-POCIUS, J.E., CAIN, J.A. & LACHMANN, P.J. (1983) Generation of three different fragments of bound C3 with purified factor I or serum: II: Location of binding sites in the C3 fragments for factors B and H, complement receptors and bovine conglutinin. *J. exp. Med.* **158,** 334.

ROSS, G.D., YOUNT, W.J., WALPORT, M.J., WINFIELD, J.B., PARKER, C.J., RANDALL FULLER, C., TAYLOR, R.P., MYONES, B.L. & LACHMANN, P.J. (1985) Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptor) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J. Immunol.* **135,** 2005.

SCATCHARD, G. (1949) The attraction of proteins for small molecules and ions. *Ann. NY Acad. Science.* **51,** 660.

SHERWOOD, T.A. & VIRELLA, G. (1986) The binding of immune complexes to human red cells: complement requirements and fate of the RBC-bound IC after interaction with human phagocytic cells. *Clin. exp. Immunol.* **64,** 195.

SIEGEL, I., LIV, T.L. & GLEICHER, N. (1981). The red cell immune system. *Lancet* **ii,** 556.

STEINBERG, A.D. (1985) The management of systemic lupus erythematosus. In: *Textbook of Rheumatology.* (Ed. W.M. Kelly, E.D. Harris, S. Ruddy & C.B. Sledge) 2nd ed, p. 1098. W.B. Saunders, Philadelphia.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arth. Rheum.* **25,** 1271.

VIK, D.P. & FEARON, D.T. (1985) Neutrophils express a receptor for iC3b, C3dg and C3d that is distinct from CR1, CR2 and CR3. *J. Immunol.* **134,** 2571.

WALPORT, M., MACKWORTH-YOUNG, C., ROSS, G.D., HOGG, N., HUGHES, G.R.V. & LACHMANN, P.J. (1985) Family studies of erythrocyte complement receptors type I levels: reduced levels in patients with SLE are acquired not inherited. *Clin. exp. Immunol.* **59,** 547.

WILSON, J.G., WONG, W.W., SCHUR, P.H. & FEARON, D.T. (1982) Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. *New Engl. J. Med.* **307,** 981.

WILSON, J.G., RATNOFF, W.B., SCHUR, P.H. &

Fearon, D.T. (1986) Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. *Arthritis Rheum.* **29,** 739.

Wilson, L. & Friedkin, M. (1967) The biochemical events of mitosis. II. The *in vivo* and *in vitro* binding of colchicine in grasshopper embryos and its possible relation to inhibition of mitosis. *Biochemisty* **6,** 3126.