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The requirement of localized, CR2-mediated, alternative pathway activation of complement for covalent deposition of C3 fragments on normal B cells

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SUMMARY

We have shown previously that normal B cells share, with Epstein–Barr virus-transformed and malignant B cells, the ability to activate the alternative pathway (AP) of complement *in vitro*, resulting in the deposition of C3 fragments on the cell surface. Complement receptor type 2 (CR2, CD21) has been implicated directly as the site for formation of an AP convertase, which provides nascent C3b for deposition at secondary sites on the B-cell surface. In the present study, we have examined C3 fragment deposition *in vitro* in more detail by (1) assessing the importance of locally generated C3b for the deposition process, (2) investigating whether CR2 is the sole requirement for conferring AP activation capacity on a cell, and (3) determining whether CR2's function, as an AP activator, has different structural requirements from ligand binding. Increasing the availability of native C3, by increasing the serum (NHS) concentration, resulted in enhanced C3 fragment deposition on the B cells, whereas use of factor I-depleted NHS, which showed massive fluid phase C3 conversion during the incubation, diminished the deposition. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting of untreated and hydroxylamine-treated lysates from B cells, after *in vitro* activation, revealed that the majority of C3 fragments (primarily iC3b and C3dg) had been covalently bound to the cell surface. Transfection of COS cells with wild-type CR2 or a deletion mutant lacking 11 of the molecule's 15 homologous domains, but retaining the ligand-binding site, revealed that expression of intact CR2 conferred a 12-fold increase in AP-activating capacity on these cells, while no increase in AP activity was apparent on cells transfected with the mutant CR2.

INTRODUCTION

The capacity of Epstein–Barr virus-transformed B cells, and malignant B cells from chronic lymphocytic leukaemia (CLL) patients, to activate the alternative pathway (AP) of the complement system, by a complement receptor type 2 (CR2)-dependent mechanism, is well established.[1–5] Subsequent studies have revealed that the degree of AP activation at the surface of various B-cell lines correlates directly with the number of CR2 expressed per cell,[6] and that activation is associated with covalent attachment of C3b fragments to CR2.[7] The initial assumption that this activation by diseased B cells might constitute a mechanism for their clearance has been challenged recently by the finding that normal peripheral blood B cells were not only capable of *in vitro* AP activation, but also showed evidence of *in vivo* activation, in the form of deposited C3d on the freshly isolated cells.[8] In contrast to the B-cell lines, AP activation on normal B cells could be blocked using Fab fragments of a polyclonal rabbit anti-human CR2 antibody (Ab), but not with a monoclonal antibody (mAb) (OKB7) directed against the ligand-binding site of the receptor.[8] Thus, it appeared that separate regions of the molecule were involved in ligand binding and AP activation, respectively. Recently, however, a role for the binding site has been indicated on the basis of its ability to bind hydrolysed C3 (C3i) and assemble a stable convertase similar to the transient form generated in the fluid phase.[9,10]

Taken together, the findings mentioned above indicate that CR2 may be involved directly in the formation of a localized AP convertase that supplies nascent C3b fragments for deposition at secondary sites on the B-cell surface. In the present study, we have chosen to investigate the mechanism of B-cell AP activation further by (1) assessing the importance of local generation of C3b for the deposition process, (2) investigating whether CR2 is the sole requirement for conferring AP activation capacity on a cell, and (3) determining whether CR2's function, in this respect, has different structural requirements from those for ligand binding.

The first objective was approached both by increasing C3 availability, through increasing the serum concentration used

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Abbreviations: AP, alternative pathway of complement; RFI, relative fluorescent intensity; SFEq, soluble fluorescent equivalents.

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for AP activation, and by depleting the serum of factor I, thereby inducing large-scale C3b production in the fluid phase, remote from the B-cell surface. The latter objectives were tackled by transfecting COS cells with cDNA encoding either intact huCR2 or a deletion mutant, PP, consisting of the two N-terminal domains (short consensus repeats; SCR), which contain the ligand-binding site, one hybrid domain, consisting of parts of SCR3 and SCR14, and the C-terminal SCR (SCR 15), as well as the transmembrane and cytoplasmic segments.[11]

MATERIALS AND METHODS

Buffers

PBS–BSA comprised phosphate-buffered saline (PBS; pH 7·4) supplemented with 0·05% NaN_3, 0·5% bovine serum albumin (BSA) and 10 mM EDTA. VBS comprised 4 mM Na-barbiturate, 145 mM NaCl, pH 7·4. VB^{2+} comprised VBS supplemented with 0·8 mM $MgCl_2$ and 0·15 mM $CaCl_2$. Mg-EGTA comprised 0·5 M EGTA and 110 mM $MgCl_2$ in water. Tris–barbital buffer comprised 73 mM Tris, 24 mM Diemal, 3·4 mM calcium lactate, 0·05% azide, pH 8·6. TBS comprised 10 mM Tris, 140 mM NaCl, 0·05% azide, pH 7·4. Trypsin–EDTA comprised 0·05 g trypsin and 0·02 g EDTA in PBS. RPMI-1640 was supplemented with 50 mM Tris–HCl, pH 7·5. DEAE–dextran (10 mg/ml) was used in TBS with a final concentration 250 μg/ml in transfection mixture.

Antibodies

Rabbit anti-human C3c and C3d Ab (reactive with native C3, C3b, iC3b, and C3c or C3dg, respectively) were purchased as unconjugated or fluorescein isothiocyanate (FITC)-conjugated IgG preparations (DAKO, Glostrup, Denmark). Fab fragments of these were generated by papin digestion and affinity chromatography on protein A–Sepharose, to remove undigested IgG and Fc fragments. The FITC/IgG (F/P) ratio for the cleaved Ab was determined, as described elsewhere,[12] to be 1·03 : 1 for anti-C3c and 0·95 : 1 for anti-C3d. As a B-cell marker, we used R–phycoerythrin(R-PE)-conjugated mouse anti-human CD19 mAb (Dako). Polyclonal sheep anti-human factor I was purchased from The Binding Site (Birmingham, UK). The anti-CR2 mAb, HB135 and OKB7, either unlabelled or FITC-conjugated, were used to detect CR2 on COS cell transfectants. The F/P ratios for FITC–HB135 and –OKB7 were 2·7 and 2·5, respectively. Goat $F(ab')_2$ anti-mouse IgG Tricolor (Caltag Laboratories, Burlingame, CA) was used in conjunction with the unlabelled anti-CR2 mAb. FITC-conjugated HB8592 (anti-CR1 mAb) and murine IgG2a were used as specificity and isotype-matched negative controls.

Cells and sera

Human peripheral blood lymphocytes (PBL), from healthy laboratory staff, were prepared by lysis of whole blood with isotonic NH_4Cl and were washed twice with PBS and once with VBS. Tonsillar mononuclear (TM) cells were prepared from tonsils provided by the Otorhino-Laryngological Department, Odense University Hospital, Denmark. The cells were prepared by forcing minced tonsils through a fine wire mesh into PBS and centrifugation of the resulting cell suspension on Lymphoprep (Nycomed Pharma). Normal human serum (NHS) was prepared as a pool from three normal AB Rh^+ donors. Factor I-depleted serum was prepared by passing NHS through a column of anti-factor I coupled to CNBr-activated Sepharose (bed volume 2 ml), equilibrated with VBS and 10 mM EDTA. Three to four millilitres of NHS, containing EDTA (final concentration 10 mM), was applied to the column and eluted with VBS. A 2–3-ml fraction, consisting of essentially undiluted serum, was collected on ice, and dialysed against 2 l VBS overnight at 4°. The efficiency of factor I depletion of the NHS was tested by crossed immunoelectrophoresis (CIE).

Transfection of COS cells

Plasmids containing either wild-type CR2 cDNA, or the CR2 deletion mutant PP (kindly donated by Dr J. Ahearn, John Hopkins University, Baltimore, MD,[11] were transfected into COS cells using the DEAE–dextran procedure, as described elsewhere.[13,14] Briefly, 3×10^6 COS cells, harvested with trypsin–EDTA, were resuspended in 3 ml RPMI containing Tris–HCl and washed twice in complete DMEM and once in RPMI. DEAE–dextran (75 μl) was added, followed by DNA (6 μg/ml) resuspended in TBS, or TBS alone (mock transfected cells). After mixing gently, cells were incubated for 2 hr at 37°, in a 5% humidified CO_2 atmosphere, washed twice and cultured for 48–72 hr to allow expression of CR2, after which they were harvested for analysis.

Activation of the AP

Cells were incubated with either 25% NHS or 25% factor I-depleted serum for 30 min at 37° in the presence of 20 mM EDTA or 20 mM EGTA/4·4 mM $MgCl_2$. The reaction was stopped by addition of EDTA to a final concentration of 20 mM and transfer to ice.

Measurement of C3 fragment deposition and CR2 expression

Blood leucocytes were washed three times with PBS–BSA and incubated with FITC-conjugated anti-C3c or -C3d and R-PE–anti-CD19 for 2 hr on ice, in the presence of excess human IgG (5 mg/ml; KABI, Pharmacia, Stockholm, Sweden). COS cell transfectants were incubated with FITC-conjugated anti-C3c together with unconjugated HB135 or OKB7 for 2 hr on ice. Samples were washed and stained with a secondary antibody, goat $F(ab')_2$ anti-mouse IgG Tricolor, for 1 hr at room temperature. Expression and quantification of CR2 on COS cell transfectants was measured by incubating washed cells with 1 μg/ml of the FITC-conjugated mouse anti-CR2 mAb, HB135 or OKB7, for 2 hr on ice. Mock transfected cells were treated similarly.

Flow cytometry

Cellular fluorescence was measured in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and analysed using the Lysis II program. COS cells' autofluorescence was compensated for in channels FL1 and FL3, on the basis of the signal in FL2. FL1 (FITC) intensities were converted to soluble fluorescein equivalents (SFE) with the help of Quantum 25 standard FITC beads (Flow Cytometry Standards Corp., San Juan, CA) and CR2 numbers were calculated from the known specific fluorescence activities of the probing mAb.[12] Analysis of AP activity was performed using quadrant statistics on the FL1–FL3 dot plots of morphologically gated cells. The AP activity was determined as the difference in FL1 signal between cells incubated in NHS–Mg-EGTA and in NHS–EDTA. The activities of the transfectants

were calculated as the amount of anti-C3c (measured in SFE) bound per expressed CR2, after deduction of the AP activity seen with the mock-transfected cells, and the activity of the PP CR2 (PPCR2) mutant was then determined as a percentage of that for wild-type CR2 (WtCR2), i.e.

$$\text{Activity of PPCR2} = \frac{(\text{AP act.}^{PP} - \text{AP act.}^{Mock}) \times \text{wtCR2/cell}}{\text{PPCR2/cell} \times (\text{AP act.}^{WT}) - \text{AP act.}^{Mock}} \times 100.$$

Solubilization of cell membranes from TM cells

TM cells (10^8) were subjected to *in vitro* AP activation as described above. We have shown previously that $84 \pm 16\%$ of the anti-C3d-reactive fragments, deposited in this way, are located on TM B cells.[15] The TM membranes were solubilized by suspending the cells in 1 ml PBS containing 1% Triton X-100, 20 mM PMSF, 5 mM iodoacetamide, 5 mM cyclocaprone, 5 mM EDTA and 10 U Trasylol for 40 min at 0°. The solubilisate was then centrifuged for 20 min at 10 000 ***g*** and the supernatant harvested. Portions of the supernatant were then treated with 1 M hydroxylamine (HA) for 1 hr at room temperature before further analysis.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting

SDS–PAGE. Slab gels (65 × 83 mm) were prepared as described by Laemmli[16] with the following modifications. The stacking gel employed was 4% (w/w) acrylamide, while the resolving gel was a 4–20% gradient, with N,N,N′,N′-tetramethyl diamine (TEMED) concentrations of 0·146% (v/v) and 0·032%, respectively. Reduction of the TM cell solubilisate was performed with 0·055 M dithiothreitol, instead of 2-mercaptoethanol, and the samples were alkylated with 0·12 M iodoacetamide. Electrophoresis was carried out with an initial current of 20 mA per gel.

Western blotting. The proteins were transferred to polyvinylidine difluoride membranes (Immobilon, Millipore, Hedehusene, Denmark) by electrophoresis for 450 volt-hours, in a 12·5 mM Tris/96 mM glycine buffer, pH 8·4, containing 20% ethanol. The membrane was blocked by incubation in Tris-buffered saline containing 0·1% Tween-20 and 0·5 M NaCl for 15 min at room temperature. The lanes were excised and incubated for at least 1 hr at room temperature with 20 μg of the primary Ab (rabbit anti-C3c, -C3d or, as negative control, normal rabbit IgG) in TBS containing 0·01% Tween-20 and 0·5 M NaCl. Alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (0·3 μg/ml Dako) in the same buffer was used as the secondary reagent. The strips were fixed with 0·2% glutaraldehyde for 10 min and then incubated for 15 min in 0·1 M ethanolamine, pH 9·0, before immersion in the chromogen, Nitro Blue Tetrazolium (0·1 mg/ml), 5-bromo-4-chloro-2-indolyl phosphate (50 μg/ml) and $MgCl_2$ (4 mM) (all from Sigma Chemicals, St Louis, MO) in 0·1 M ethanolamine, pH 9·0. The reaction was stopped by washing the strips in distilled water when an acceptable staining had been achieved. The data presented refer only to bands showing specific reactivity with the anti-C3 antibodies.

Crossed immunoelectrophoresis

Electrophoresis was performed in 1% agarose, 3% polyethylene glycol (PEG) gels in Tris-barbital buffer, pH 8·6. In the investigation of C3 split product generation, the supernatants from *in vitro* cellular AP activation were run in the first dimension gel at 10 V/cm for approximately 1 hr, and then overnight at 2·5 V/cm against anti-C3c antibody (final concentration, 7 mg/l) in the second dimension. Factor I-depletion was measured using the same conditions by running the affinity absorbed serum against anti-factor I at a final concentration of 0·15 mg/ml in the second dimension.

Statistical analysis of data

Confidence intervals were calculated for the experiments, where factor I's influence on C3-fragment deposition was measured, using Student's *t*-test. Inhibition was considered significant if zero was not included in the confidence interval.

RESULTS

The requirement for local generation of nascent C3b

In order to assess the importance of locally generated C3b for the covalent deposition of C3 fragments, human PBL were incubated with various concentrations of either NHS or factor I-depleted NHS containing Mg/EGTA. As might be expected, increasing the serum, and thereby C3 concentration, resulted in a substantial, progressive enhancement of C3 fragment deposition on B cells, up to 75% serum, while leading to only modest increases in deposition on the other leucocyte subpopulations (Fig. 1). The majority of the C3 fragments deposited on the B cells was shown, by SDS–PAGE and Western blot analysis of tonsil cell lysates, to be covalently bound as a mixture of iC3b and C3dg fragments (Fig. 2). Thus, in the absence of HA treatment, the only C3 components detected in the reduced samples were the anti-C3c-reactive β-chain (74 000 MW) and C-terminal portion of the iC3b-derived α-chain (42 000 MW), whereas, after HA treatment, prominent anti-C3d-reactive bands, corresponding to the N-terminal segment of the α-chain from iC3b and C3dg (the 68 000 and 38 000 MW bands, respectively; Fig. 2) were also seen.

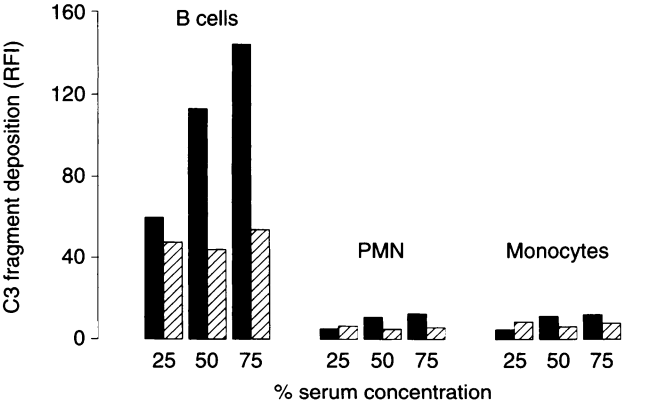

Figure 1. Effect of serum concentration and factor I depletion on the degree of C3 fragment deposition on B cells, monocytes and PMN following *in vitro* AP activation. The cells were incubated with either NHS (solid bars) or factor I-depleted NHS (striped bars), at final concentrations of 25%, 50% and 75%. FITC-conjugated anti-C3c antibodies were used to detect the deposited C3 fragments. The values are given as net relative fluorescent intensities (RFI), after subtraction of RFI observed in the NHS/EDTA controls. The data shown are from one of two experiments performed.

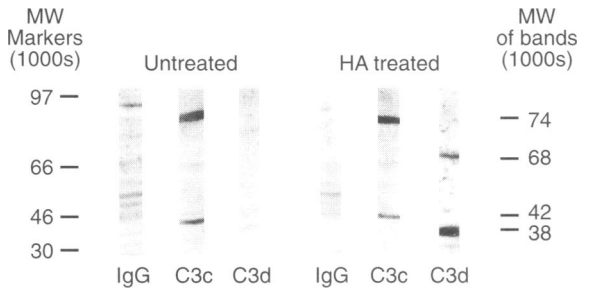

Figure 2. SDS–PAGE and Western blots of a TM cell lysate, following *in vitro* AP activation. A portion of the lysate was treated with HA and both the treated and untreated samples were reduced before electrophoresis. The blots were probed with rabbit anti-human (hu) C3c, anti-huC3d and normal rabbit IgG. The positions of the molecular weight markers are shown on the left hand side of the figure and the molecular weights of the bands described in the text are shown on the right.

Factor I depletion of NHS resulted, at 25% serum concentration, in a significant reduction in the binding of C3 fragments to the B cells, but not to either polymorphonuclear (PMN) cells or monocytes (Fig. 1 and Table 1). In addition, the enhancement in deposition, seen with increasing NHS concentration, was completely abrogated by removal of factor I. CIE of the supernatants from cells treated with 25% factor I-depleted NHS, revealed that reduced deposition occurred despite the fact that substantial amounts of C3-split products were being generated in the fluid phase (Fig. 3).

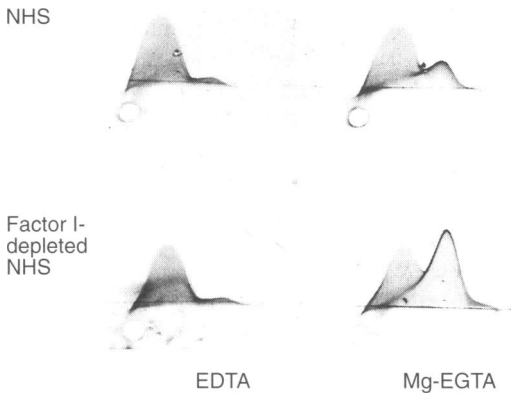

Figure 3. Detection of native C3 and C3 fragments in the fluid phase after *in vitro* AP activation on normal B cells, with NHS or factor I-depleted NHS. The supernatants were tested by CIE with anti-C3c Ab in the second dimension. Native C3 is seen in the precipitation arc closest to the origin, and C3 fragments in the arc with high mobility. Complement source and chelator used in the respective samples are as indicated.

Transfection of COS cells with intact CR2 induces AP activation capacity

COS cells, transfected with wtCR2 and the mutant PP, were incubated with 25% NHS containing Mg-EGTA or EDTA for 30 min at 37° and then probed for C3 fragment deposition. A representative experiment is illustrated in Fig. 4. Incubating the wild-type transfectant in the presence of Mg-EGTA NHS resulted in an approximately 12-fold increase in the subsequent binding of anti-C3c to $CR2^+$ cells (Fig. 4a), compared to the binding arising from treatment of the $CR2^-$ mock transfectant (Fig. 4c). When the corresponding dot-plots for the CR2 deletion mutant PP were compared, only a 1·2-fold increase was seen on the transfected cells (Fig. 4b, c). When the data from seven experiments performed with wild-type and PP CR2 transfectants were analysed, the relative AP-activating capacity of PP was found to be only 5±29% of that for wild-type (Table 2).

Table 1. The inhibition of C3 fragment deposition on PBL subpopulations, following AP activation, when factor I is removed from the serum

	% inhibition of C3 fragment deposition, as measured with			
	Anti-C3c		Anti-C3d	
Cell type	Mean ± SD*	95% CI†	Mean ± SD	95% CI
B cells	36 ± 17	19–53	38 ± 13	25–51
Monocytes	−30 ± 48‡	−81–21	−1 ± 32	−35–33
Granulocytes	−2 ± 31	−35–31	25 ± 17	8–43

*Data from six experiments.
†CI, confidence interval.
‡Negative values indicate enhancement.

DISCUSSION

In the present study, we have demonstrated that the extent of covalent deposition of C3 fragments on the B cells, following *in vitro* AP activation, is dependent on the availability of native C3 in the B cells' immediate surroundings. The covalent nature of C3 fragment attachment to normal B cells was established by SDS–PAGE and Western blot analysis of tonsil cell lysates, following *in vitro* AP activation. Treatment of the lysate with hydroxylamine (HA), followed by reduction, resulted in two distinct bands reactive with anti-C3d, corresponding to the C3dg fragment (41 000 MW band, Fig. 2) and the N-terminal end of the α-chain from iC3b (68 000 MW). These fragments were detected in only trace amounts in the untreated lysates, suggesting that the majority were covalently bound to the B cells. Detection, with anti-C3c, of the β-chain of C3 (75 000 MW) and a portion of the α-chain (43 000 MW), not recognized by anti-C3d, irrespective of HA treatment, is consistent with the presence of covalently bound iC3b fragments on the tonsil cell. The failure to identify an α-chain fragment, corresponding to C3b, suggests that only a small amount of the C3 is incorporated as C3b into AP convertases, the majority being deposited at unprotected secondary acceptor sites, where it is degraded by factor I.

The dependence of fragment deposition on the generation of nascent C3b in the B cell's immediate vicinity was demonstrated by (1) raising the serum, and thereby C3, concentration, resulting in a dose-dependent increase in C3 fragment deposition, and (2) depleting the serum of factor I, leading to a fall in C3 fragment deposition at the B-cell surface despite massive

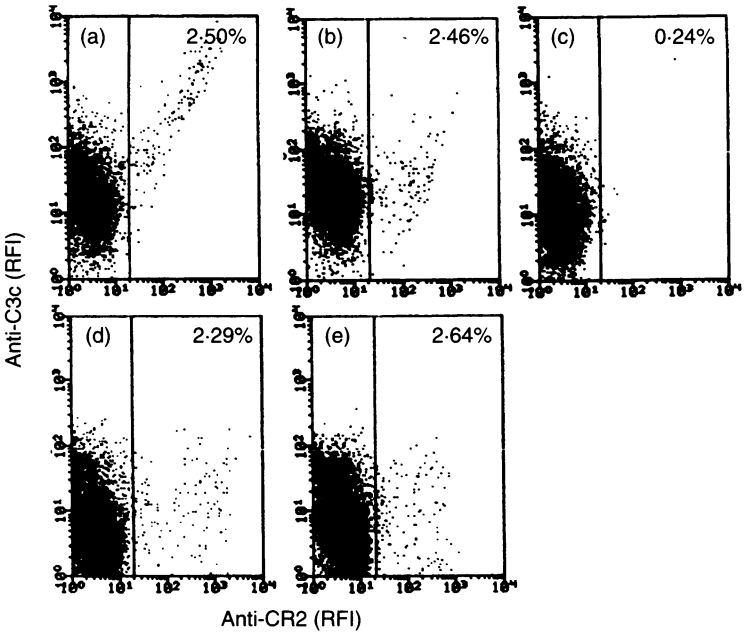

Figure 4. Dot-plots of C3 fragment deposition (FL1) versus CR2 expression (FL3) for COS cells transfected with wild-type CR2 cDNA (a and d) and PP CR2 cDNA (b and e). A dot-plot of mock transfected cells (control) is shown in (c). The cells were probed with FITC anti-C3c after incubation with NHS containing Mg-EGTA (a, b and c) or EDTA (d and e). The mean C3 deposition in RFI units for the CR2-positive cells (right-hand fields) in (a, d, b and e) is 1171, 34, 124 and 26, respectively, while the corresponding value for CR2-negative cells (left-hand field) in the mock transfectant control is 102. A representative experiment out of seven performed.

Table 2. Relative activities of wild-type CR2 and PP mutant CR2 as activators of AP

Receptor	Mean transfection efficiency	Mean number of CR2 per cell ($\times 10^{-5}$)	Relative activity per CR2 (%)*
wtCR2	4.1 (1.0–7.0)†	6.8 ± 1.1‡	100
PPCR2	1.7 (0.3–4.9)†	2.7 ± 1.1‡	5.4 ± 29.4‡

*The relative activity (AP-activating capacity) of PPCR2, compared with wtCR2 was calculated on the basis of the mean C3 fragment deposition per CR2 (see the Materials and Methods).

†Values in parentheses are the range for all experiments.

‡Mean ± SD of the values determined from seven experiments.

production of C3b in the fluid phase (Fig. 3). The latter observation is consistent with the fact that the exposed thioester group in nascent C3b, which is responsible for covalent attachment to acceptor molecules, has a very short lifetime in an aqueous environment (about 60 μs)[17] and is unlikely to engage in nucleophilic attack on such targets outside a radius of 30 nm from the site of its formation.

Given the unique ability, amongst leucocytes, of B cells to activate the AP, we deemed it appropriate to (1) test whether CR2 was the sole requirement for induction of this activity, and (2) see whether it was possible to distinguish structurally between the receptor and AP-activating functions of CR2. To these ends we transfected COS cells with cDNA coding for wild-type CR2 and for the deletion mutant, PP. This mutant contains only the homology regions, SCR1, 2, 3/14 and 15, as well as the transmembrane and cytoplasmic domains, and has previously been characterized as binding the CR2 ligand, C3dg, and reacting with OKB7, a mAb with specificity for the ligand-binding site.[18] Transfection of COS cells with wild-type CR2 resulted in a 12-fold increase in the AP-activating capacity of the cells, indicating that this, indeed, is the only B cell-specific glycoprotein required to generate AP activity. Transfection with the mutant, PP, resulted in the expression of a gene product that was recognized by the mAb, OKB7, and thus, by inference, contained the ligand-binding site. Cells expressing this mutant displayed, however, virtually the same C3 fragment uptake, following *in vitro* AP activation, as mock transfected cells (Table 2). Thus, it would appear that the capability of CR2 to act as a protected site for the formation of a stable AP convertase is heavily reliant on the portion missing in the mutant molecule, which may be presumed to fulfill at least one of three functions: provision of an acceptor site for nascent C3b, direct protection of the C3b from interaction with CR1 or factor H, or as an extended structure holding the convertase remote from the membrane regulators, DAF and MCP. This finding does not exclude the possibility that the ligand-binding site contributes to the generation of an AP convertase, but indicates clearly that its presence, alone, is not sufficient.

The data presented here demonstrate that the selective

deposition of C3 fragments on B cells, both *in vivo* and *in vitro*, arises from the formation of an AP convertase on CR2, presumably at a locality distinct from the receptor's ligand-binding site. There are, however, a number of questions that remain to be resolved. The first is whether the ligand-binding site plays a role in generating the AP convertase. It has been reported that the hydrolysed form of C3 (C3i) is a ligand for CR2[9] and that C3i, bound in this fashion, can form a convertase with factor B,[10] analogous to the transient AP convertase formed in the fluid phase. The presence of such a convertase would undoubtedly enhance the chance of nascent C3b reaching a protected acceptor site on CR2, where it could form a stable AP convertase. Evaluation of the ligand-binding site's importance, in this regard, will require investigation of CR2 mutants that lack this portion of the molecule. Secondly, since the majority of the fragments on B cells are breakdown products of C3b, and are likely to be secondary deposits, at unprotected acceptor sites, of fragments generated by the CR2 convertase, it would be of interest to identify other membrane structures acting as targets for C3 fragment deposition. Given that the deposition range, around the convertase, is very limited (see above), the most probable targets would be either those that are found in great abundance on the B cell (e.g. mIg, MHC class I and II, CD11a/18 (LFA-1α), CD45RA/RB and CD50 (ICAM-3),[19] or glycoproteins that form non-covalent associations with CR2; namely, CD19,[20] CD35 (CR1)[21] and CD23 (FcεRII).[22] Studies are in progress, in our laboratory, to characterize these secondary acceptor molecules on normal B cells. The final question is that of the phenomenon's biological function. It is now well established that the complement system plays an important role in the development of a primary humoral immune response,[23–26] and that this role is, in large part, exerted via CR2, by virtue of the receptor's association with the signal glycoprotein, CD19,[20] which augments signalling via the antigen receptor.[20,27,28] The potential influence of C3 fragments deposited in modulating B-cell responses, by forming cross-links between C3dg-bearing acceptor molecules and CR2, is currently under investigation in our laboratory.

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