Characterisation of the rat and mouse homologues of gC1qBP, a 33 kDa glycoprotein that binds to the globular 'heads' of C1q

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Abstract gC1qBP is a 33 kDa glycoprotein that binds to the globular 'heads' of C1q. We have cloned cDNAs encoding the rat and mouse homologues of gC1qBP. Comparison of the cDNA-derived amino acid sequences of gC1qBP reveals that either of the rodent sequences is 89.9% identical to the reported human sequence. Recombinant rat gC1qBP binds avidly to human C1q. gC1qBP mRNA is abundantly expressed in every rat and mouse tissue analysed. Rat mesangial cells synthesise gC1qBP, but do not express gC1qBP on the cell surface. In rat serum, gC1qBP is present at low levels.
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Key words: gC1qBP; C1q; Complement; Receptor

1. Introduction

C1q is the recognition subunit of the first component of complement. In addition to its role in the activation of the classical pathway, C1q elicits various receptor-mediated biological functions such as enhancing phagocytosis [1,2], neutrophil superoxide production [3], Ig synthesis [4,5], chemotaxis [6], cell adhesion [7], and the regulation of platelet function [8]. All types of peripheral blood cells, except erythrocytes, bind C1q [9]. C1q binding sites have also been found on endothelial cells [10], fibroblasts [11,12], smooth muscle cells, and epithelial cells [13]. Several binding molecules and potential receptors for C1q have been described: a cell surface receptor of 60 kDa isolated from tonsil lymphocytes [14]; a cell surface receptor of 100 kDa isolated from U937 cells [15]; and a glycoprotein of 33 kDa that binds to the globular 'heads' of C1q [16]. The 60 kDa C1q receptor (termed cC1qR or collectin receptor) binds to the collagenous region of C1q and to the collagenous region of the collectin proteins lung surfactant protein A (SP-A), mannose binding lectin (MBL), CL43, and conglutinin [17,18]. Also the 100 kDa monocyte C1q receptor binds to the collagenous region of C1q and the collectins MBL and SP-A [19,20]. Characterisation of cC1qR by peptide sequence analysis [21] revealed that cC1qR is a membrane-associated form of calreticulin (CaR), a highly conserved abundant calcium-binding protein [22]. Further evidence for the identity of cC1qR with CaR was provided by the localisation of a C1q binding site in CaR [23]. A cDNA sequence has been reported for the monocyte C1q receptor of 100 kDa indicating that this molecule is a novel protein furnished with a transmembrane anchor [24].

A 33 kDa glycoprotein (gC1qBP or gC1qR) which binds specifically, and with high affinity, to the globular 'heads' of C1q was first reported by Ghebrehiwet et al. [16]. Recombinant gC1qBP was shown to inhibit complement activation in human serum. gC1qBP also binds to the heparin binding, multimeric form of vitronectin and the ternary vitronectin-thrombin-antithrombin III, but not to the monomeric, uncomplexed form of vitronectin in plasma [25]. Moreover, gC1qBP was shown to bind to H-kininogen and to factor XII [26,27]. gC1qBP is identical to a HeLa cell protein (p32 or SF2) discovered by Krainer et al [28,29] and initially thought to be a pre-mRNA splicing factor. However, subsequent work by the same group casts serious doubt on this theory [30]. Here we report the primary structure of the rat and mouse homologues of gC1qBP, their mRNA expression, their cellular localisation, and C1q binding activity.

2. Materials and methods

2.1. cDNA cloning and sequencing
Proprietary λZAPII rat macrophage, λZAPII rat liver, λZAPII rat brain cortex and λZAP mouse liver cDNA libraries were purchased from Stratagene, Cambridge, UK. Isolated λZAP clones were converted into pBluescript SK⁻ plasmid clones by in vivo excision, following the supplier's protocol. Sequencing of both strands was carried out by the dideoxynucleotide chain termination procedure using [α-^{35}S]dATP and the T7 Sequencing reagent kit (Pharmacia, Uppsala, Sweden).

2.2. Ribonucleic acid extraction and Northern blot analysis
Total RNA was isolated by the method of Chirgwin et al. [31]. 15 μg of total RNA, or 2 μg of poly(A)⁻ RNA, was separated on formaldehyde-containing 1.2% agarose gels. Northern blot analyses were carried out following standard methods [32]. The cDNA insert of clone RB7.12 was labeled with [α-^{32}P]dCTP using the random priming method [32]. To validate Northern blot results, the filters were rehybridised with a α-^{32}P-labeled β-actin cDNA probe [33].

2.3. Prokaryotic expression of recombinant rat gC1qBP
Recombinant rat gC1qBP was expressed as a thioredoxin fusion protein in *Escherichia coli* using plasmid pTrxfus (Invitrogen BV, Leek, The Netherlands). Clone RB7.12 was used as a template for PCR using primer RG*Xba*I (5'-GTCTAGACCCCGCGATGCTCCC-TCTG-3') with an *Xba*I site arranged to allow in-frame cloning into pTrxfus and primer BS*Srf*I (5'-GGATCGCCCGGGCTGCAGG-3', a pBluescript primer modified to include a *Srf*I site). The PCR product was digested with *Srf*I/*Xba*I and subcloned into pTrxfus. Expression was performed according to the manufacturer's protocol. The fusion

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protein was affinity purified by binding to Thiobond resin (Invitrogen).

2.4. Antibodies

An NZW rabbit was injected s.c. with 100 μg of recombinant rat gC1qBP thiofusion protein in 500 μl of PBS plus 1 volume incomplete Freund's adjuvant. Three further immunisations were done at fortnightly intervals before the rabbit was bled. Anti-human gC1qBP polyclonal antibodies were prepared as described in [16].

2.5. Solid phase binding assay

An ELISA was devised to determine the binding of biotinylated rat gC1qBP thiofusion protein to C1q. Proteins were biotinylated according to established methods [35]. Wells of a Nunc PolySorp microtitre plate (Gibco-BRL Life Technologies) were coated with increasing concentrations of human C1q in 15 mM Na_2CO_3, 35 mM $NaHCO_3$ pH 9.6 and incubated for 2 h at RT. After coating, the plate was washed three times with 20 mM Tris-Cl, 150 mM NaCl, 0.05% Tween 20, 1 mg/ml BSA, blocked for 2 h at RT with 300 μl/well of the same buffer and washed again. 100 μl of a 1 μg/ml solution of either biotinylated rat gC1qBP thiofusion protein or, for controls, biotinylated thioredoxin peptide, was added to each well and incubated for 2 h at RT. The plate was washed three times and developed with ExtraAvidin alkaline phosphatase followed by the chromogenic substrate Sigma 104 pNPP (Sigma-Aldrich Company, Poole, UK). Absorbance was measured at 405 nm.

2.6. Cells and FACS analysis

Rat mesangial cells (RMC) were isolated and maintained as previously described [36]. Cultured, adherent RMC were detached using 20 mM EDTA/PBS, incubated for 30 min in PBS, 1% BSA and 1% normal rat serum at 4°C and washed. Cells were exposed for 1 h at 4°C to either 1/500 diluted polyclonal rabbit anti-rat gC1qBP or, for controls, 1/500 diluted pre-immune serum. Cells were washed, incubated for 1 h at 4°C with FITC conjugated goat anti-rabbit IgG and analysed using a FACScan (Becton Dickinson, Cowley, UK). Intracellular gC1qBP was assayed as above, except that cells were permeabilised with PBS containing 1% (v/v) saponin (Sigma-Aldrich) for 45 min at RT and washed, prior to incubation with the primary antibody.

3. Results

Human gC1qBP cDNA [16] was labeled with [α-^{32}P]dCTP and used to screen rat macrophage and liver cDNA libraries. Positive plaques were picked and the pBluescript SK$^-$ plasmid rescued from the λZAPII phagemid vector by in vivo excision.


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Rat        MLPLLRCVPR ALGAAATGLR ASIPAPPLRH LLQPAPRPCL RPFGLLSVRA  -21
Mouse      MLPLLRCVPR SLGAAS.GLR TAIPAQPLRH LLQPAPRPCL RPFGLLSVRA  -22
Human      MLPLLRCVPR VLGSSVAGLR AAAPASPFRQ LLQPAPRLCT RPFGLLSVRA  -24
           ********** **   ***   **    *  ********* **********
                                                1
Rat        GSARRSGLLQ PPGSCA..CA .AALHTEGDK AFVEFLTDEI KEEKKIQKHK   27
Mouse      GSARRSGLLQ PPVPCA..CG CGALHTEGDK AFVEFLTDEI KEEKKIQKHK   27
Human      GSERRPGLLR PRGPCACGCG CGSLHTDGDK AFVDFLSDEI KEERKIQKHK   27
           ** ** ***  *   ** * *   *** *** *** ** *** *** ******

Rat        SLPKMSGDGE LEVNGTEAKL LRKVAGEKIT VTFNINNSIP PTFDGEEEPS   77
Mouse      SLPKMSGDWE LEVNGTEAKL LRKVAGEKIT VTFNINNSIP PTFDGEEEPS   77
Human      TLPKMSGGWE LELNGTEAKL VRKVAGEKIT VTFNINNSIP PTFDGEEEPS   77
           ******  * ** ******* ********* ********** **********

Rat        QGQKAEEQEP ELTSTPNFVV EVTK.TDGKK TLVLDCHYPE DEIGHQDEAE  126
Mouse      QGQKAEEQEP ERTSTPNFVV EVTK.TDGKK TLVLDCHYPE DEIGHEDEAE  126
Human      QGQKVEEQEP ELTSTPNFVV EVIKNDDGKK ALVLDCHYPE DEVGQEDEAE  127
           **** ***** * ******** **        ** **** ** ** * ****

Rat        SDIFSIKEVS FQTTGDSEWR DTNYTLNTDS LDWALYDHLM DFLADRGVDN  176
Mouse      SDIFSIKEVS FQATGDSEWR DTNYTLNTDS LDWALYDHLM DFLADRGVDN  176
Human      SDIFSIREVS FQSTGESEWK DTNYTLNTDS LDWALYDHLM DFLADRGVDN  177
           ****** *** ** ** *** ********** ********** **********

Rat        TFADELVELS TALEHQEYIT FLEDLKSFVK SQ                    208
Mouse      TFADELVELS TALEHQEYIT FLEDLKSFVK NQ                    208
Human      TFADELVELS TALEHQEYIT FLEDLKSFVK SQ                    209
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Fig. 1. Alignment of the cDNA-derived peptide sequences of rat, mouse and human gC1qBP. Residues identical in all three species are indicated by asterisks. Three potential *N*-glycosylation sites are underlined.
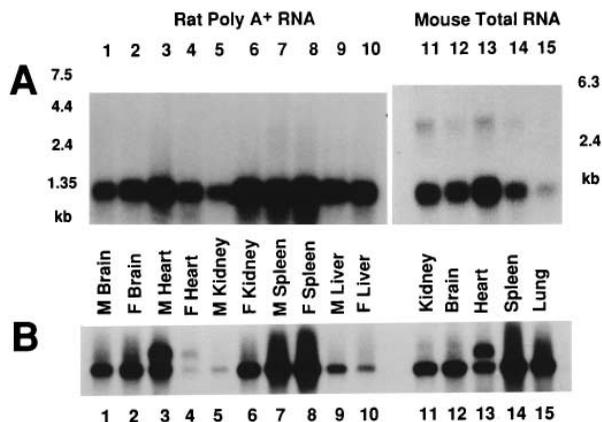

Fig. 2. Northern blot analysis of rat and mouse tissue RNA preparations. Total RNA was isolated from the mouse tissues shown. Poly(A)$^+$ RNA was prepared from the male (M) and female (F) rat tissues indicated. RNA was separated on formaldehyde-containing agarose gels, transferred to nylon membranes and probed with ^{32}P-labeled RB7.12 cDNA (A). The position of RNA size standards is shown left, and the size and position of rRNAs in the total RNA preparations is shown right. The Northern blot shown in A was subsequently hybridised with a α-^{32}P-labeled β-actin cDNA probe (B).

Sequencing of the two longest clones revealed extensive homology with the published human sequence. As these clones were incomplete at their 5' ends, they were used to screen a rat brain cortex cDNA library, yielding clone RB7.12 (1124 bp). RB7.12 contains a 5' untranslated region of 45 bp followed by an 834 bp open reading frame and a 3' untranslated region of 245 bp. A putative polyadenylation initiation signal (aataaa) is located 19 bp upstream of the poly(A)$^+$ tail. Analysis of the three different rat cDNA clones revealed complete sequence identity.

RB7.12 was used to screen a mouse liver cDNA library. One clone, MG2 (1127 bp), encompasses the entire coding region. Sequencing showed that, like RB7.12, MG2 contains an open reading frame of 834 bp. The sequence data are available from the EMBL database, under accession numbers AJ001102 (rat) and AJ001101 (mouse).

The cDNA-derived amino acid sequences are compared in Fig. 1. Human gC1qBP is reported to be synthesised as a pre-pro protein of 282 residues, of which the first 73 amino acids comprise a 13 residue leader peptide followed by a 60 residue hydrophobic stretch. The mature protein begins at residue 74, a leucine, which we have designated residue 1. Both rodent gC1qBP pre-pro proteins are 4 residues shorter than the human sequence. None of the gC1qBP proteins contain classical transmembrane or GPI anchor domains.

There is a striking degree of cross-species conservation: rat and mouse share a degree of identity of 97.6%, and either of the rodent sequences is 89.9% identical to the human amino acid sequence. Differences are mostly confined to the N-terminus. The C-terminus contains a region of 60 residues which is identical in all three species. 3 potential N-glycosylation sites (underlined) are entirely conserved. Total RNA from mouse tissues and poly(A)$^+$ selected RNA from rat tissues was analysed by Northern blotting. A single gC1qBP mRNA species of ~1.3 kbp is abundantly expressed in all tissues, except in mouse lung, where a weaker signal was detected (Fig. 2A). Northern blots were normalised using a β-

actin cDNA probe (Fig. 2B). Note that while preparations 4 and 5 (female rat heart and male rat kidney, respectively) contain less RNA than the other preparations, the weaker signals from rat livers RNAs are consistent with previous reports that liver has ~ 2.5 times less β-actin mRNA than muscular tissues like heart [34]. In other experiments various human cell lines – including Raji (B lymphoblastoid), K562 (erythroblastoid), T47D (breast carcinoma), HT29 (adenocarcinoma), TP410 (neuroblastoma), TP379 (glioblastoma) and HL60 (promyelocytic leukaemia) – were all shown to express a single ~ 1.3 kbp gC1qBP mRNA transcript (data not shown).

To confirm that RB7.12 encodes a C1q-binding protein, and to assess the extent of cross-species conservation, we prepared recombinant rat gC1qBP thiofusion protein. On Western blots, this fusion protein strongly stained with anti-human gC1qBP antisera, whereas no staining was observed for the thioredoxin fusion-partner alone (data not shown). We next devised a solid phase binding assay to determine whether rat gC1qBP binds to human C1q. A microtitre plate was coated with human C1q (1–10 μg/ml) as described in Section 2. As shown in Fig. 3, recombinant rat gC1qBP thiofusion protein binds to human C1q in a saturable, concentration-dependent manner, whereas thioredoxin peptide alone shows nominal binding. Rat mesangial cells (RMC) were shown to express gC1qBP mRNA by Northern blot analysis. Western blot analyses using polyclonal rabbit antibodies raised against recombinant rat gC1qBP revealed the presence of gC1qBP in cell lysates and culture supernatants of cultured RMC (not shown). FACS analyses demonstrated that gC1qBP is not expressed on the surface of RMC (Fig. 4A), but is abundantly expressed intracellularly as seen after permeabilisation with saponin (Fig. 4B). Western blots and ELISA showed that gC1qBP is present in rat serum at low levels (data not shown).
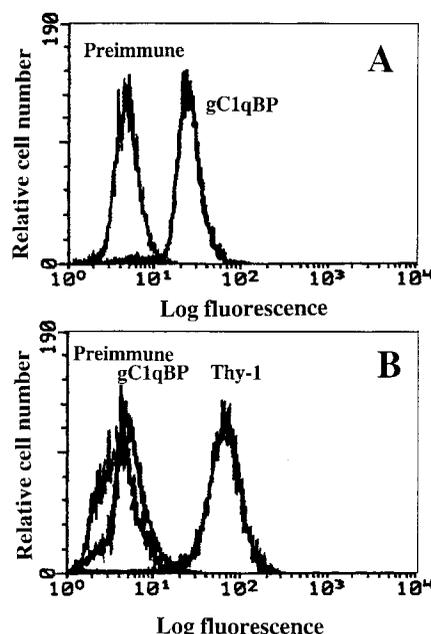
Fig. 4. gC1qBP is synthesised by RMC, but is not expressed on the cell surface. Saponin permeabilised (A) or untreated (B) RMC were incubated with either anti-rat gC1qBP polyclonal antibodies, pre-immune rabbit serum (negative control) or anti-Thy-1 antibodies (positive control). The monoclonal mouse anti-rat Thy-1 antibody (IgG2a) was raised as previously described [38]. Antibody binding was assessed by addition of a FITC conjugated goat anti-rabbit secondary antibody.

4. Discussion

Characterisation of the rat and mouse homologues of gC1qBP revealed that the primary structure of this molecule is highly conserved. Moreover, we have shown that rat gC1qBP avidly binds to human C1q. The 1.3 kbp gC1qBP mRNA is abundantly and constitutively expressed in every tissue and cell line examined. Some confusion surrounds the precise cellular location of gC1qBP. In the first report on this molecule [16], confocal scanning laser microscopy results indicated that gC1qBP is located in the vicinity of the plasma membrane in Raji cells. It was therefore suggested that this molecule may represent a novel type of cell surface receptor for C1q binding to the globular 'heads' of C1q (gC1qR) [16]. However, van den Berg et al. [37] were unable to detect gC1qBP on Raji cells using FACS analysis unless the cells had first been permeabilised with saponin. Likewise, another group reported that gC1qR is mainly localised in the vesicular fraction, but not on the surface of endothelial cells [38]. We have shown that in cultured RMC gC1qBP expression is intracellular and gC1qBP detectable in the cell culture supernatant, but absent on the cell surface. Combined, these results strongly reinforce the assertion that gC1qBP is an intracellular protein, and contradict the previous suggestion that it might act as a functional cell surface receptor for C1q. We therefore favour the term gC1qBP rather than gC1qR for this C1q binding protein.

The discovery of gC1qBP in rat serum, with its proven C1q binding capacity and its ability to inhibit C1 activation, suggests a possible complement regulatory role.
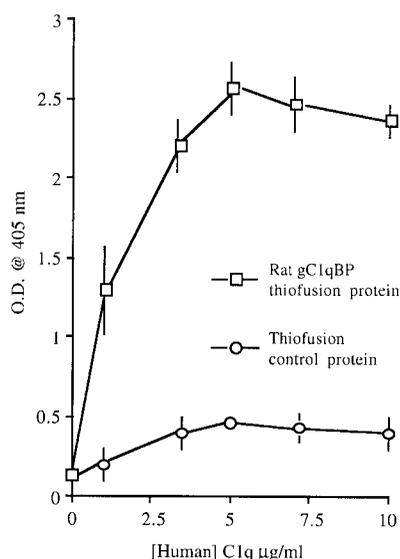

Fig. 3. Recombinant rat gC1qBP binds to human C1q. A microtitre plate was coated with human C1q at the concentrations indicated (abscissa). Biotinylated proteins were added and binding assessed as described in Section 2. Measurements were carried out in triplicate (see error bars).

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