Comparative Functional Evolution of Human
and Mouse CR1 and CR2

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The complement cascade is regulated by a series of proteins that inhibit complement convertase activity. These regulatory proteins, most of which possess binding sites for C3b and/or C4b, can be roughly divided into two groups, one that controls inappropriate complement convertase activity on the surface of cells and another that controls convertase activity on immune complexes in serum. In this review we focus upon the structural and functional comparisons of the CR1 and CR2 proteins of man and mouse. A single gene encodes these proteins in the mouse whereas the human requires two. The analysis of mice lacking the CR1/CR2 proteins demonstrates the requirement of these proteins for the regulation of complement convertase activity within lymphatic tissue immune complexes that is not efficiently controlled by other membrane-bound or serum regulatory proteins.

Complement regulatory proteins

The complement pathway is a series of serum proteins that functions to tag nonself proteins and microbes for phagocytic uptake and destruction. This activation pathway has, by necessity, evolved a series of serum and membrane bound proteins whose primary goal is to help regulate the pathway, allowing for the targeting of foreign Ags but protecting normal self tissue and cells from deleterious attack. The primary amplification step of the complement cascade is the generation of the activated C3 protein (through the classical, alternative, or lectin activation pathways). The initial cleavage of C3 by these various convertases generates C3a (which is a potent anaphylatoxin recognized by the C3a receptor) and C3b (which may form a covalent thiol-ester bond to substrate). C3b can also join with the C3 convertases to function as a C5 convertase, releasing the anaphylatoxin C5a and the C5b protein. C3b can be further degraded into smaller, inactive forms known as iC3b and C3dg by the serine protease factor I (fI), which requires cofactor help (4). Each of these C3b cleavage products can maintain its bond to substrate (and is recognized by a series of receptors) but cannot participate within C3 or C5 convertases.

The C3 complement convertases are the targets of many of the complement regulatory proteins. For example, decay acceleration factor (DAF) enhances the decay of complement convertases by binding to C3b. Another set of proteins, typified by membrane cofactor protein (MCP), serves to facilitate fI cleavage of C3b into the smaller, inactive forms. An additional protein, known in sub primates as Crry, possesses both MCP and DAF functions. These complement regulatory proteins are all membrane bound and are relatively small (45,000–70,000 Da), suggesting that their primary function is to protect the membrane of that cell from complement-mediated damage.

Convertase regulation is also accomplished through a number of serum proteins. C4 binding protein (C4BP) regulates the classical and lectin complement pathways by serving as a cofactor for fI-mediated degradation of C4b proteins within those C3 and C5 convertases as well as by accelerating the decay of these convertases. The serum protein factor H (fH) helps regulate the alternative pathway by aiding in fI-mediated cleavage of C3b as well as by destabilizing the convertase. fH is also implicated, in association with the acute phase protein C-reactive protein (CRP), of solubilizing immune complexes (15); alleles of fH that do not bind CRP are linked to predisposition to macular degeneration (16–18). The site of action of C4BP and fH are within the blood stream as well as connective tissue, especially during events in which vascular leakage is promoted by responses to infectious agents.

Another set of cellular complement receptor regulatory proteins are typified by much larger membrane-bound proteins. Primate CR1 ranges in size from 190,000 Da to nearly 300,000 Da and serves as a cofactor for the cleavage of activated C3b and C4b into their inactive forms. It is also a phagocytic receptor of macrophages and neutrophils for complement-bound immune complexes. Primate CR1 is also a key player in the immune adherence phenomenon in which complement-bound immune complexes are first bound to erythrocytes, transported to the spleen and liver, and then removed...
from the erythrocytes for phagocytosis in the liver and spleen (22–24). Both human and mouse CR2 are ~150,000 Da and bind the degraded C3d and C3d,g forms of C3 (25–27). Because CR2 only binds the inactive forms of the C3 protein, it has minimal complement regulatory functions but functions primarily as a member of the B cell coreceptor complex. CR3 and CR4 are integrin phagocytic complement receptors that bind the inactive but partially degraded form of C3, iC3b, bound to immune complexes, leading to internalization of the complexes (28).

One intriguing aspect of the membrane-bound complement regulatory proteins MCP, DAF, Cry, CR1, and CR2 and the soluble complement regulatory proteins fH and C4BP is that they have evolved from a common structural and functional domain, the short consensus repeat (SCR), an ~60-aa sequence with internal disulfide bonds (also known as a Sushi domain or a complement control protein domain) (29). The shorter regulatory proteins possess four to five SCR while the CR1 and CR2 proteins possess 14 or more. These domains make up the entirety of the extracellular sequences of these regulatory proteins and yet have evolved differential specificities for binding to the various ligands, i.e., C4b, C3b, C3d, etc. The numbers, types, and expression patterns of genes that encode this group of complement regulatory proteins are also variable yet redundant. For example, the human has a single DAF gene, yet the mouse appears to possess two functional copies of DAF genes, albeit with restricted tissue expression and structure (30). Additionally, the human MCP protein (CD46; also known as the measles receptor) (31) is expressed by a wide variety of cells while the rodent gene is preferentially expressed in testis (32), regulating sperm acrosome reaction (33). But perhaps the most intriguing difference between the human and mouse complement regulatory proteins is in the structure and expression of the genes encoding the CR1 and CR2 proteins.

**Comparative evolution of murine and human CR1**

The canonical subprimate CD21/Gr2 gene produces two proteins, CR1 (~200,000 Da) and CR2 (~145,000 Da), via alternative splicing for an additional six protein N-terminal domains for the CR1 protein (Fig. 1) (34, 35). This CR1 protein can bind both C4b and C3b and possesses cofactor activity for fI-mediated cleavage (27). In the mouse, the Gr2 gene (encoding CR1 and CR2) is expressed by B cells and follicular dendritic cells (FDCs). Both of these cell types produce both the CR1 and CR2 proteins via alternative splicing; no stimulation has been observed to preferentially splice to either the CR1 or the CR2 form (our unpublished data). It is likely that the duplication of Crry sequences within the Gr2 gene allowed for the creation of the subprimate CR1 protein. A survey of CR1 proteins from nonprimate mammals (by genomic analysis of rat, cow, and dog) encoded from the alternatively spliced CD21/Gr2 gene allowed for the creation of the subprimate CR1 protein. A survey of CR1 proteins from nonprimate mammals (by genomic analysis of rat, cow, and dog) encoded from the alternatively spliced CD21/Gr2 gene allowed for the creation of the subprimate CR1 protein.

**Comparative analysis of C3b/C4b mouse and human regulatory proteins**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Size (Da)</th>
<th>Function</th>
<th>Cell Type Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Crry</td>
<td>65,000</td>
<td>Receptor: decay C convertase and cofactor for C3 cleavage</td>
<td>All cells including erythrocytes</td>
</tr>
<tr>
<td>Cr2</td>
<td>CR2</td>
<td>145,000</td>
<td>Receptor: C3d, C3dg, C3d-bound complexes</td>
<td>B cells and FDC</td>
</tr>
<tr>
<td>Cr1</td>
<td>CR1</td>
<td>200,000</td>
<td>Receptor: C3b, C4b-bound complexes and cofactor for C3 cleavage</td>
<td>B cells and FDC</td>
</tr>
<tr>
<td>Daf1.2</td>
<td>Daf 1.2</td>
<td>70,000</td>
<td>Receptor: decay C convertase</td>
<td>All cells including erythrocytes</td>
</tr>
<tr>
<td>CD46</td>
<td>Mcp</td>
<td>60,000</td>
<td>Receptor: sperm acrosome reaction</td>
<td>Testis</td>
</tr>
<tr>
<td>Cfh</td>
<td>Factor H</td>
<td>150,000</td>
<td>Serum: cofactor for C3 cleavage and decay C3 convertase</td>
<td>Hepatocytes (serum)</td>
</tr>
<tr>
<td>C4BP</td>
<td>C4bp</td>
<td>570,000</td>
<td>Serum: cofactor C4 cleavage</td>
<td>Hepatocytes (serum)</td>
</tr>
<tr>
<td>Human</td>
<td>CR1</td>
<td>180–280,000</td>
<td>Receptor: C3b, C4b-bound complexes, cofactor for C3 cleavage and decay C convertase</td>
<td>T/B cells, phagocytes FDC, erythrocytes, glomerular podocytes</td>
</tr>
<tr>
<td>CR2</td>
<td>CR2</td>
<td>145,000</td>
<td>Receptor: C3d, C3dg, C3d-bound complexes</td>
<td>B cells and FDC</td>
</tr>
<tr>
<td>DAF</td>
<td>DAF</td>
<td>70,000</td>
<td>Receptor: decay C convertase</td>
<td>All cells including erythrocytes</td>
</tr>
<tr>
<td>MCP</td>
<td>MCP</td>
<td>60,000</td>
<td>Receptor: cofactor, C3 cleavage</td>
<td>Nucleated cells</td>
</tr>
<tr>
<td>CFH</td>
<td>Factor H</td>
<td>150,000</td>
<td>Serum: cofactor C3 cleavage, C3 convertase decay</td>
<td>Hepatocytes (serum)</td>
</tr>
<tr>
<td>C4BP</td>
<td>C4BP</td>
<td>570,000</td>
<td>Serum: cofactor C4 cleavage</td>
<td>Hepatocytes (serum)</td>
</tr>
</tbody>
</table>

**Figure 1.** Comparative structure of the mouse and human CR1 and CR2 proteins and genes. A. Comparison of the functional domains of the CR1, CR2, and Cry proteins. Red blocks denote common sequences used to build the Cry and human CR1 proteins and the N-terminal domains of the mouse CR1 protein. Green blocks represent common sequences used to build the Crry and human CR1 proteins and the N-terminal sequences of the mouse CR1 protein. Blue blocks represent common sequences used within the human and mouse CR2 proteins. T, Transmembrane; C, cytoplasmic domains. B. Differential genome organization and alternative splicing of the mouse Gr2 and Cr2 genes and human CR2 and CR1 genes present on mouse and human chromosome 1. The light pink box within the human Cr2 gene denotes those Cr1-like sequences that are not included within mature Cr2 transcripts. The blue box of the mouse and human Cr2/CR2 genes encodes the signal sequence. The black boxes represent T and C domains. This figure is not drawn to scale nor does it reflect the full exon/intron splicing complexity of these genes.
The evolutionary bottleneck of sacrificing the lacking Crry are embryonic lethal unless the mother lacks C3 functions of DAF and MCP to help keep cellular surfaces clear CR1 protein on the surface of erythrocytes and the combined creation of coding CR1-like domains are present within the human CR2 gene between the exons encoding the signal sequence and the first domains of the CR2 protein but are not incorporated into functional transcripts (36). Thus at the step(s) in evolution that separated primates from other species, the CR2 gene lost the ability to encode the CR1 protein from the CR2 gene.

Humans do express a protein, CR1, that recapitulates many of the structural domains (and presumed functions) of subprimate Cr2-derived CR1. The human CR1 protein is made up of the same SCR domains organized into groups of seven SCR, termed long homologous repeats (LHR), that share a very high degree of homology to one another and hence are indicative of a very recent amplification event in the genome (20, 37). There are a variety of CR1 alleles in the human population, each consisting of genes possessing three, four, five, or six of these LHR domains, that encode proteins of ~190,000—300,000 Da (38, 39). These LHR possess C3b and C4b binding activity, and the CR1 protein functions as cofactor for fI-mediated cleavage of these substrates (19). Alternative splicing of other prime CR1 genes may also provide a wider array of protein products from this gene (40).

Sequence homology analysis of the human CR1 gene indicates that it was derived from the constituents of the Crry gene found in subprimates (Fig. 1) (37). The sequences and functions of both gene products are very similar, except that the Crry protein (60,000 Da) is much smaller than the human CR1 isoforms. This common derivation is consistent with the localization of these genes in relation to the Cr2/CR2 genes in chromosome 1 of mouse and man. In the mouse, the 5’-end of the Crry gene is <10,000 bp from the 3’-end of the Cr2 gene. In the human, the CR1 gene is also immediately adjacent to the 3’-end of the CR2 gene although, due to the duplication of sequences, the genomic footprint of the prime CR1 gene is much larger than that of the subprimate Crry gene. The same overall structure of the human CR1 gene is also found in the chimpanzee, rhesus, and baboon genomes. Other than the mouse, the dog genome is the most complete of the subprimate genomes for the delineation of genes encoding the complement regulatory proteins. The dog genome possesses a single Cr2-like gene immediately adjacent to two Crry-like genes flanked by two MCP-like genes.

The Crry gene was sacrificed during primate evolution for the creation of CR1; primates lack this gene. The impact of the loss of the Crry gene product during this expansion event would have been critical if not for the continued expression of the new CR1 protein on the surface of erythrocytes and the combined functions of DAF and MCP to help keep cellular surfaces clear of potentially damaging complement convertases. This latter point is especially critical during fetal development in that mice lacking Crry are embryonic lethal unless the mother lacks C3 (41). The evolutionary bottleneck of sacrificing the Crry gene to create the larger primate CR1 protein must have had an impact upon the reproductive success of the animal.

Function for human CR1

The study of the function of the human CR1 protein is intriguing in that it has been implicated in a number of biological roles. The evolution of the human CR1 gene from a Crry-like precursor to recreate the lost Cr2-derived CR1 protein resulted in the expansion of the coding sequences and the limitation of cell surface expression. Virtually all cells of the mouse express Crry (42), yet mouse CR1 (and CR2) is only expressed on B cells and FDCs (although some reports suggest limited T cell expression) (43–45). The human CR1 gene has a wider cell expression profile than that of mouse Cr2-CRI (including B cells, FDC, macrophages, neutrophils and RBCs) but less so than Crry, indicating a contraction of expression in many cell types.

Human CR1 expression on circulating erythrocytes has been demonstrated to be critical for binding complement-bound immune complexes (46) and facilitating the transfer of such complexes to, for example, the Kupfer cells of the liver. Kupfer cells possess the CR1g protein, an Ig domain-containing complement receptor for C3b- and iC3b-bound complexes that has been identified as the critical receptor on mouse and human Kupfer cells for the internalization of complement-bound immune complexes (47). The role of mouse Crry on erythrocytes is analogous to that of human CR1, suggesting that the presence of a receptor for complement-bound immune complexes on erythrocytes is critical. Additionally, human CR1 has demonstrated phagocytic capacity on the surface of neutrophils and macrophages for complement-bound immune complexes, although its functions are highly redundant to those of the integrin CR3 and CR4 receptors that bind and internalize C3b- and iC3b-bound complexes (48). CR1 has also been implicated as a receptor for another two opsonins, C1q and the mannann-binding lectin (49, 50).

The human CR1 protein is a potent regulator of complement activation. Soluble recombinant CR1 proteins have been created and used to control complement activation during heightened complement activation (51–53). The ability of the CR1 proteins to bind to both C3b and C4b allows for the control of classical, alternative, and lectin pathway C3/C5 convertases. Soluble murine Crry proteins have also been used in analogous models to control complement activation (54, 55).

The human CR1 gene in the human population exists in a variety of alleles derived by the amplification of groups of exons. Although these numerous alleles could be due to the genetic instability of the recent evolutionary expansion of highly homologous sequences, it may also be that there are benefits for having CR1 proteins of varying sizes on the surface of B cells and FDCs in the spleen and on erythrocytes for the binding of C3-bound complexes. Various alleles of human CR1 have been linked to resistance to malaria and susceptibility to lupus, suggesting differential environmental or genetic stresses could perpetuate CR1 polymorphisms (56, 57).

Requirement for a CR1 protein

Our primary model is that the primate Cr2 gene, in losing the ability to create both the CR1 and CR2 proteins, generated an immunological stress that could only be alleviated by recreating a new CR1 protein via the use of the Crry-like sequences. An alternative scenario can be proposed where the amplification of the Crry gene in primate lineages was actively selected to create much larger proteins. The generation of a large CR1 protein with multiple C3b/C4b binding sites (as seen in the human) might have created a more efficient protein for complement control than the subprimate Cr2-derived CR1 protein. If the
alternative scenario is correct, then the Gz2-derived CR1 protein would have been left as an appendix of the regulatory family, easily lost. Both models, however, predict that a CR1-like protein is critical for the animal. What role could the CR1 protein play in host defense that would make its presence so indispensable and not be covered by the redundant roles of the other membrane-bound and serum complement regulatory proteins? To address this question, the mouse model system is informative.

Analyses of the mouse CR1/CR2 proteins have implicated them in a variety of functions. These proteins expressed by FDCs are not phagocytic but instead, like FDC Fc receptors, serve to hold Ag on the surface of such cells for generating a strong Ab response and for affinity maturation of B cells (58–60). Both human and mouse CR2 have been implicated in the transport of immune complexes. In human cell lines, immune complexes bound to B cell CR2 are transferred to THP-1 monocytic cells (61). In vivo, PE-labeled immune complexes are first captured by macrophages in the subcapsular sinus, transferred to follicular B cells, and subsequently deposited on the surface of FDCs, a function that is dependent upon complement receptor expression on B cells (62). A similar transfer between marginal zone B cells and FDCs in the spleen is observed upon in vivo tracking of CR1/2 mAb (63). These studies imply a role for complement receptors (at least CR2) on both B cells and FDCs in the recognition, processing, and retention of Ags.

The mouse CR1 protein can bind both C4b and C3b complexes whereas mouse (and human) CR2 binds C3dg-bound complexes. CR1 and CR2 on murine B cells form complexes with a coaccessory activation complex including CD19, CD81, and the frags/lifim proteins (the mouse LEU13 equivalents) (64–67). As seen for human CR2, the coligation of the mouse BCR and complement-bound Ag via CR2/CD19 complexes reduces the threshold of activation for B cell responses, allowing for the activation of Ag-specific, naive B cells with limiting amounts of foreign Ag (68). The human and mouse CR2 proteins have been linked to a variety of intracellular signaling pathways, including a membrane phosphoprotein p53, nucleolin-mediated regulation of PI3K (69), and Ag internalization and processing responses (70). The mouse CR1 protein possesses the same C-terminal protein sequence as the mouse CR2 protein, such that association with and activation through CD19 should be equivalent for both proteins. Human CR1, however, does not associate with CD19 on the surface of B cells and cannot participate in this process (65). Thus, it is unlikely that B cell intracellular signaling is a required function of the human and mouse CR1 proteins.

Mice lacking CR1/CR2 expression on FDC and B cells via introduced mutations in the Gz2 gene show depressed T cell-dependent and -independent Ab responses to low-dose immunizations, specific depression of Ag-specific IgG3 following immunization, a heightened sensitivity to Streptococcus pneumoniae infections and diffuse unorganized germinal centers (71–73). IgG3-dependent feedback enhancement of Ab responses was also impaired in CR1/2-deficient mice (74). Analysis of animals expressing lower levels of shorter CR1/CR2 proteins (hypomorph expression) (71, 75) shows that Ag-specific B cells in these mice are eliminated from the germinal centers, presumably through impaired B cell coreceptor signaling (76, 77). Finally, deficiencies in the CR1/2 proteins are implicated in the production of autoreactive Abs in certain autoimmunity models (59, 78, 79). The preceding observations have assumed that the loss (or depressed response) of B cell functions was due to the lack of intracellular signaling via the CR2/CD19 complex or signaling alone.

An alternative explanation for some, if not all, of the phenotypes associated with the Gz2-deficient and hypomorphic mice could be due to the loss of the extracellular complement regulatory component of the mouse CR1 protein. The size of this cell surface protein indicates that its function could be to regulate C3/C5 convertase activity on extracellular splenic immune complexes held away from the cell surface and out of the controlling grasp of the Cry and MCP proteins. This regulatory function of the CR1 protein might be particularly critical on the surface of FDCs, in that they do not phagocytize immune complexes that could possess active complement convertases. The absence of the complement convertase regulatory properties of the CR1 protein in the spleen could result in heightened local C3 and C5 convertase activity, leading to increased cellular death and enhanced inflammatory responses due to the release of the anaphylatoxins C3a and C5a.

Recent analyses have shown that the spleen from a naive Gz2-deficient animal is in a heightened state of inflammatory activation compared with a naive wild-type (WT) spleen (80). The expression of a number of inflammatory marker genes are elevated in the Gz2-deficient spleen, and these elevated expression profiles can be brought back to WT levels by depleting serum C3 via treatment of the animals with cobra venom factor (CVF) (which consumes serum C3) or by blocking C3a receptor function. The spleens of the Gz2-deficient animals also possess elevated numbers of immature myeloid regulatory cells compared with WT spleens, which is consistent with a chronic state of inflammation in the organ (81, 82). The elevated expression of these inflammatory genes can be returned to WT levels by treating Gz2-deficient animals with anti-Gr-1 mAb, which transiently eliminates virtually all neutrophils from the spleen.

One of the consistent hallmarks of the Gz2-deficient animals has been the relative loss of Ag-specific IgG3 isotypes. When Gz2-deficient animals were treated with CVF to deplete C3 at the same time as immunization with a T cell-independent Ag, the levels of Ag-specific IgG3 were returned to the same levels as WT animals treated with CVF. Thus, the presence of C3 in the Gz2-deficient animal is partly responsible for the depressed IgG3 production, implicating the byproducts of complement consumption (presumably C3a and/or C5a) as inhibiting the production of this isotype (83, 84).

The preceding data indicated that the removal of C3 relieved the inflammatory stress of the Gz2-deficient animal. Thus, the loss of the Gz2-derived proteins in the animal can potentially provide two sets of deficiencies, those due to the loss of CR1/CR2 membrane signaling (either with or without CD19 involvement) and those due to the loss of the complement regulatory component of the CR1 protein. It will be of interest to examine a number of the other known phenotypes associated with the Gz2-deficient animals to determine whether they are due to the heightened inflammatory state of the animal or the lack of B cell signaling. At least two animal models can be envisioned that could tease these two effects apart. The first would be to create an animal that only possesses the CR2 protein and does not express the alternatively spliced CR1 protein. Such animals would thus lose the complement regulatory contributions...
of the CR1 protein but would leave intact the complement-bound Ag B cell signaling pathway (thus creating for the mouse the equivalent of the human CR2 gene). Alternatively, replacing the sequences encoding the transmembrane and cytoplasmic domains of the CR2 gene with those from a nonsignaling protein (such as the MHC class I protein that abrogates the formation of the CR2/CD19 complex) (65) would leave the complement regulatory and receptor functions of the CR1 and CR2 proteins intact but would eliminate the ability of these proteins to effectively provide intracellular signaling. Both of these animal models are currently under analysis (our unpublished data).

The regulation of complement activation has focused upon blocking convertase formation on cell surfaces via the proteins DAF, MCP and Cry and disrupting active convertases in the serum using the soluble regulators C4BP and fH. Together, these sets of proteins should be able to control complement activation pathways in the body. However, as shown in Fig. 2, the analysis of the CR2-deficient mouse suggests that lymphoid tissues require an additional level of convertase control. The deposition of immune complexes in lymphoid tissues places convertases too far from the cell surface for DAF, MCP, or Cry to regulate, but out of the reach of the serum regulators. The large size of CR1 would allow it to modulate the activity of the complement convertases bound to such extracellular immune complexes. As depicted in Fig. 2, misregulation of complement convertases due to lack of the CR1 protein would allow for the excessive release of C3a and C5a. Although only misregulation of the alternative pathway is shown in Fig. 2, any of the pathways could be similarly affected. fH is ~150,000 Da while C4BP is much larger at ~570,000 Da. The larger size of the C4BP protein may pose a greater restriction for its exit from the circulatory system and entry into the lymphoid tissue matrix of the spleen than fH. Thus CR1 proteins (both mouse and human) may be more critical for the neutralization of C3/C5 convertases generated from the classical or lectin pathway than for the alternative pathway C3/C5 convertase.

Concluding remarks

The analysis of animals possessing engineered genetic defects has always been limited by the preconception of phenotypes and the penetrance of the defect. Apart from the vagaries associated with the techniques (85), immunological concerns of such genetically engineered animals can include the lack of the optimal test for their analysis, the potential of other proteins providing complementary and overlapping functions, and the difficulties in discriminating a specific phenotype by proteins with multiple functions. The CR2-deficient mice are illustrative of such concerns in that separating the extracellular complement regulatory effects of the deficiency from those implicit with the diminished capacity of the CD19-associated B cell signaling pathway has complicated their analysis. No doubt similar concerns exist for many such gene deficiency models, lending credence to the application of unbiased assays (such as gene expression analyses) as a complement to their initial characterization.

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Disclosures

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