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ORIGINAL ARTICLE

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Complement C2 receptor inhibitor trispanning: from man to schistosome

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Abstract Horizontal gene transfer (HGT), in relation to genetic transfer between hosts and parasites, is a little described mechanism. Since the complement inhibitor CRIT was first discovered in the human *Schistosoma* parasite (the causative agent of Bilharzia) and in *Trypanosoma cruzi* (a parasite causing Chagas' disease), it has been found to be distributed amongst various species, ranging from the early teleost cod to rats and humans. In terms of evolutionary distance, as measured in a phylogenetic analysis of these *CRIT* genes at nucleotide level, the parasitic species are as removed from their human host as is the rat sequence, suggesting HGT. The hypotheses that CRIT in humans and schistosomes is orthologous and that the presence of CRIT in schistosomes occurs as a result of host-to-parasite HGT are presented in the light of empirical data and the growing body of data on mobile genetic elements in human and schistosome genomes. In summary, these data indicate phylogenetic proximity between *Schistosoma* and human CRIT, identity of function, high nucleotide/amino acid identity and secondary protein structure, as well as identical genomic organization.

CRIT is found in the platyhelminth human parasite *Schistosoma*

Complement C2 receptor inhibitor trispanning (CRIT) represents a novel family of receptors, the first member of which was found in *Schistosoma haematobium* and, subsequently, *Schistosoma mansoni* [14]. Schistosome is a platyhelminth worm and is the causative agent of Bilharzia. Around 250 million people worldwide, mainly in tropical countries, are afflicted

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with the disease. The intermediate host is a fresh waterborne snail. The infectious stage, which is released from the snail into the water, is called the cercaria. Infection is by means of penetration of the skin by a combination of proteolytic action and vigorous movement of the cercarial tail. Upon infection and shedding of the tail, the cercaria transforms into the larval stage, termed the schistosomulum. The mature worms, which live *in copulo*, settle in mesenteric venules of the intestine or those surrounding the urinary bladder. The pathology of the disease is due to the granulomatous response against eggs which, having been shed by the female worm, become trapped in the liver, intestinal wall, spleen or other organs [4, 26].

CRIT was found by screening an adult worm cDNA library of *S. haematobium* with a 'vaccination serum' obtained from baboons that had been vaccinated with γ-irradiated cercariae. This serum was passively protective to challenge infection with *S. mansoni* cercariae. Therefore, antigens that were recognized by this serum, especially those that were found on the surface of the parasite, were deemed good vaccine candidate antigens. In such a screening, a λgt11 clone containing the CRIT cDNA was found and sequenced [14]. At this point, an affinity-purified polyclonal antibody against a 27-mer synthetic peptide based on the N-terminal putative first extracellular domain 1 (ed1) region (Fig. 1) was generated to reveal the native CRIT protein as an approximately 32-kDa protein. The CRIT mRNA transcript at 1.35 kb meant that the open reading frame (ORF) of 0.86 kb was approximately of full length, thereby confirming the size of the native protein.

The parasite's sensitivity to complement (C) is dependent on its life cycle stage. The infectious cercariae, which are free-swimming, having been shed from the intermediate host,

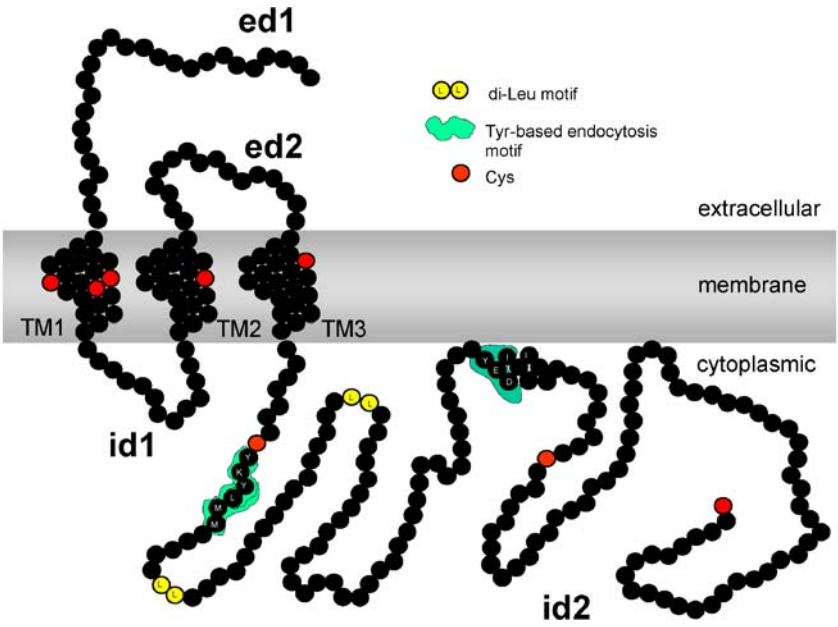

Fig. 1 Schematic topology prediction of CRIT showing N-terminal extracellular domains (ed1 and ed2), three N-terminal transmembrane domains (TM1, TM2 and TM3), two intracytoplasmic domains (id1) and the cytoplasmic tail (id2). Also indicated are tyrosine-based endocytosis motifs in id2, as well as cysteine residues shown in *red*

which is a fresh waterborne snail, are C-sensitive but become C-resistant upon infection and transformation to larval schistosomulae living within their host [6]. We found that CRIT expression at the mRNA level was greater in the C-resistant schistosomulae than in the adult worm [14], although we do not know if this translates to an increased level of CRIT expression at the protein level. Interestingly, others have found the mRNA expression of CRIT to be still higher in the eggs [11], which is not surprising as eggs, too, need to be protected en route to the environment ex vivo. The C-resistant schistosomulae and mature adult worm are able to evade complement by expressing proteins that mimic known C-regulators and by acquiring at least one of the host's C-regulatory proteins. By acquiring DAF from human erythrocytes, the adult worm can disassemble the classical and alternative pathway C3 convertases. By mimicking human CD59, the *Schistosoma* SCIP-1 protein binds the late complement components C8 and C9 of the multiple attack complex, thereby preventing C-mediated lysis[33]. CRIT is a recently described complement regulatory receptor, which appears to target the early complement pathway. By sharing antigenic determinants with the C2-binding C component C4 and by acting as a decoy C2-binding receptor, CRIT regulates C activation on the surface of schistosomulae by limiting the amount of C3 convertase formation.

Using scanning electron microscopy, we showed CRIT to be located on the surface of the many pits and channels in the tegument of the adult worm. These pits and channels (Fig. 2) are filled with host blood and, by increasing the surface area of the interface between the parasite and the host blood, aid in the quicker absorption of nutrients from the blood. It is therefore likely that part(s) of the extracellular domains of CRIT interacts with component(s) in human blood. CRIT in humans is also found on the surface—in this case on the plasma membrane of a monocytic cell, as seen in Fig. 2b.

The membrane topology of CRIT, as predicted by several algorithms, shows a molecule with an extracellular N-terminus and a cytoplasmic tail (Fig. 1). Since this is the typical topology of a type IIIb membrane protein [38] (extracellular N-terminus, several transmembrane domains and no cleavable signal peptide), we went on to find a likely ligand for this putative receptor. Besides this prediction, we had empirical data for this topology and so, by using extracellular domains (ed1 or ed2), we tried to isolate the ligand from normal human serum by affinity chromatography. This formed the basis of a simple 'receptor affinity chromatography' experiment [20] in which we arbitrarily used a synthetic peptide, chosen to be ed1, coupled to an epoxy-activated Sepharose affinity column through those amino acid side chains containing NH_2, -OH or -SH groups. Amongst the few serum proteins spe-

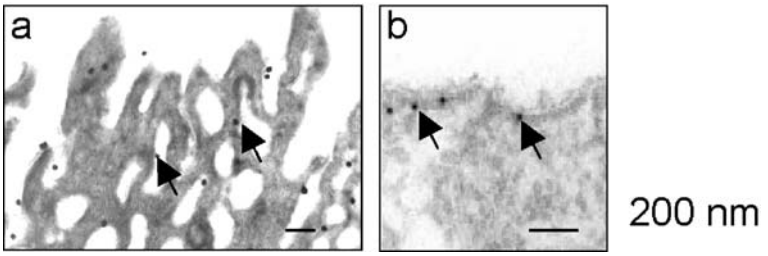

Fig. 2 Scanning electron micrographs of anti-CRIT-ed1/immunogold-labelled **a***Schistosoma haematobium* surface tegument and **b** human monocyte cell surface. CRIT protein is expressed in the surface pits and channels found in the *Schistosoma* tegument, which presents an interface with the host blood. CRIT is also found on the plasma membrane of the monocyte from where it may be able to undergo endocytosis

cifically bound to the matrix, after extensive washing and then elution from the column with low-pH glycine, a protein identified by N-terminal sequencing and confirmed by specific recognition with a monoclonal antibody was human complement C2. With this finding, our initial working hypothesis was that the C2-binding CRIT receptor could play a role in regulating the activation of CP (Classical Pathway) on the parasite surface. To this end, we and others found that the synthetic peptide CRIT-ed1 inhibited CP activation both in vitro [18, 31] and in vivo [19, 32].

However, CRIT was also found to be phosphorylated on tyrosine and to have a plethora of putative binding sites on the cytoplasmic tail or intracellular domain 2 (id2) (Fig. 1) for a host of signalling molecules, including tyrosine kinases such as Fyn. In schistosomes, such molecules are believed to play a role in cell growth and development, with a Fyn-like tyrosine kinase having been found in schistosomes [23]; this strongly suggests, as was speculated recently [15], that CRIT may play similar roles, in addition to the regulation of complement. In its cytoplasmic tail, CRIT has characteristic Tyr-X-X-Leu and Tyr-X-X-Ile motifs as well as two dileucine motifs (Fig. 1), which could be involved in receptor endocytosis that is mediated via the clathrin-coated pit pathway. Although endocytosis has not been shown in schistosomes, a homologue of a rat component of the adaptor complex that links clathrin to receptors in coated vesicles exists in *Schistosoma japonicum* [11], as does an *S. mansoni* orthologue of a *Caenorhabditis elegans* clathrin coat assembly protein [40]. *S. mansoni* also expresses a clathrin heavy-chain protein and dynamin [40], which is associated with endocytic sorting of proteins. The cytoplasmic tail of CRIT also has tyrosine-based motifs, which, if phosphorylated, could be consensus motifs for binding to SH2 domains of various cytoplasmic tyrosine kinases, including Fyn. In mammals, amongst other functions, fyn is involved in T-cell receptor (TCR) signalling, binding to the CD3–TCR complex and being activated when the TCR is crosslinked. Fyn is also involved in platelet-derived growth factor receptor mitogenic signalling and, from studies of fyn −/− mice, it participates in the differentiation of myelin-producing oligodendrocytes as well as keratinocytes. Fyn also participates in cell-adhesion-mediated signalling [34].

CRIT is a novel complement regulator in humans

Human CRIT was recently cloned from testis and pancreas cDNA [16]. This study confirmed CRIT binding to C2 by ligand blotting and flow cytometry, and addressed the mechanism by which CRIT regulates the classical pathway. CRIT expression at the protein level was looked at in various tissues. CRIT was expressed strongly in glandular epithelial cells of proliferating —but not secretory—endometrium and pancreatic islet cells (probably insulin-producing β-cells). In the kidney, where the activation of complement is believed to expedite the progression of chronic renal damage [17], CRIT (in terms of protein expression and CRIT mRNA transcription) was uniquely confined to podocyte cells within the kidney glomerulus. This constitutive expression was confirmed in a more recent investigation (to be described in greater detail elsewhere), which showed CRIT upregulation in glomeruli in membranous nephropathy. In the testis, CRIT was expressed in cells in testicular tubules attached to the basement membrane, which were presumably Sertoli cells. CRIT was also found on a range of haemopoietic cells as well as on certain endothelial cells.

When C2 is bound to CRIT-ed1 peptide [18] or native CRIT on the cell surface [16], the cleavage of C2 by C1s is inhibited. This is neither the case with the homologous C4β$^{212–232}$ peptide, based on the so-called CRIT-ed1 domain of C4 [18], nor with native C4, of course. It could be that both CRIT-ed1 and the CRIT-ed1 domain from C4 bind C2, but it is only when CRIT-ed1 binds that there is a conformational change in the structure of C2, preventing cleavage at the C1s site or a steric interference that prevents C1s-mediated cleavage. In another work reported elsewhere, we have confirmed the β-chain of C4 as possessing an important binding site for C2 and have begun to reveal the role of the vWFA1 domain of C2 in this interaction. CRIT appears to exist as a dimer, possibly a homodimer, as it is phosphorylated on tyrosine, primarily in the dimeric form (unpublished observations), and C2 binds both to the monomeric and dimeric forms, as shown in far Western blot analysis [16]. Based on this, we compared (Fig. 3a) the sequences of CRIT-H17 motifs from two CRIT molecules, CRIT-A and CRIT-B, laid contiguously with the two CRIT-H17 motifs (F^{231}–N^{240} and F^{241}–Y^{251}) comprising the C4 β-chain (F^{231}–Y^{251}). As shown in Fig. 3a there is a 58% identity and 73% similarity across the region. We have also presented a schematic model diagram of CRIT and how we might expect it to exist in the membrane as a homodimer (Fig. 3b). We also show, by comparison, the CRIT-ed1 domain in the C4 β-chain. The two CRIT-H17 motifs NH_2-FEVKKYVLPN-CO_2H and NH_2-FEVKITPGKPY-

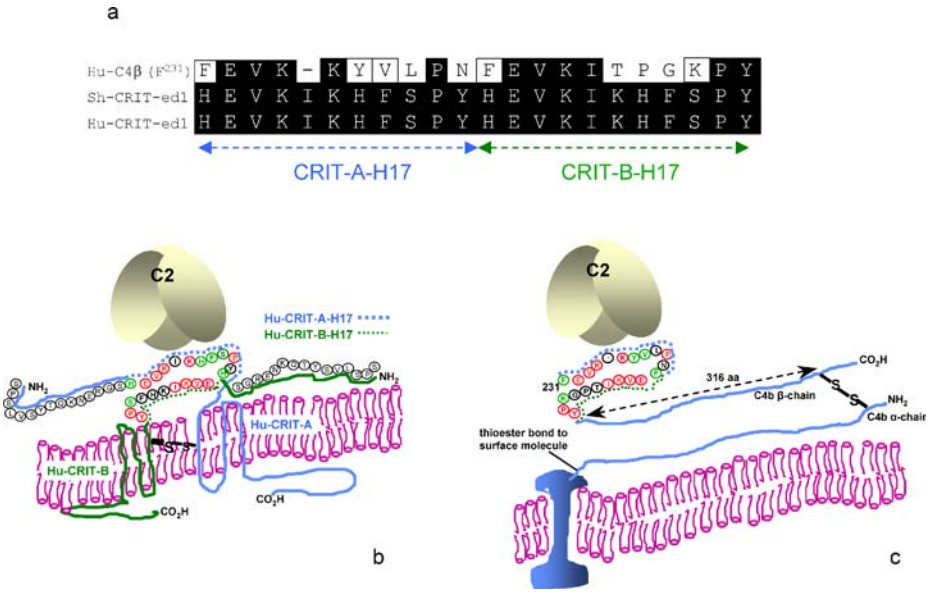

Fig. 3 Alignment of amino acid sequence of the CRIT-ed1 domain of the human C4 β-chain F^{231}–Y^{251} with *S. haematobium* and human CRIT-ed1. **a** The C-terminal 11-amino-acid CRIT-ed1, the so-called CRIT-H17 motif, is shown. In this schematic, two such motifs have been placed contiguously to represent how two such regions may come into close proximity in a CRIT homodimer made up of CRIT-A and CRIT-B. **b** A schematic of the β-chain of C4, with emphasis on the predicted C2-binding ed1 domain. **c** A schematic of two CRIT molecules constituting a homodimer in which two CRIT-H17 motifs might be brought together in such a way as to represent the equivalent of the CRIT-ed1 domain in the C4 β-chain

CO_2H, of which the CRIT-ed1 domain in the C4 β-chain is composed, are separated by a Pro^{239}-induced β-turn, and Fig. 3c illustrates how they might be expected to lie.

An interesting feature of the human CRIT molecule and its parasite orthologue, compared with the membrane regulators of complement activation (mRCA, membrane Regulators of Complement Activation), including DAF, CD59, CR1 and MCP, is its unusually long cytoplasmic tail at 163 residues, suggesting further roles besides complement regulation. In a submitted article describing the downstream molecular associations of the CRIT cytoplasmic tail in greater detail, human CRIT was found to be phosphorylated on tyrosine as was previously shown for its *Schistosoma* homologue [14]. CRIT also associates with the cytoplasmic tyrosine kinase fes (Fig. 4), which is believed to play a role in the terminal differentiation of myeloid cells. At least in certain cell types, it thus appears likely that CRIT functions in cellular signalling.

CRIT phylogeny suggests that human and *Schistosoma* CRIT are orthologous

Horizontal gene transfer (HGT), as may occur between species, can dramatically alter the evolution of eukaryotic genomes. The main driving force for this is the presence of mobile genetic elements, principally long terminal repeat (LTR) retrotransposons and retroviruses, within a genome and the later acquisition of envelope protein genes that could then render such retroviruses infectious, thereby enabling HGT.

On comparing the genetic organization of CRIT in different species, it is clear that they all possess a single exon structure. Phylogenetic analyses at both the nucleotide level (Fig. 5) and at the amino acid level (not shown) suggest that schistosome CRIT (which is absent from the nematode worm *C. elegans*) has a closer phylogenetic relationship to human CRIT than to rat CRIT. Furthermore, besides pairwise alignment showing these proteins to be 87.5% identical at the amino acid level, they have almost identical secondary structures (not shown) and share some cross-reactive epitopes [16]. The high degree of identity between human and *Schistosoma* CRIT is particularly notable in the immunologically important part of the receptor, namely the ligand binding ed1, which shows 92% identity at the amino acid level, as well as ed2 (88% identity). Whether one considers CRIT on a haematopoietic cell or on the surface of a *Schistosoma* adult worm, both molecules possess the identical function of interacting via ed1 with the same protein in normal human serum, this being human C2. The transmembrane domains also have a high degree of similarity, with the greatest sequence difference being in the N-terminal region of the cytoplasmic tail, proximal to the third transmembrane domain (TM3). This is hardly surprising as the intracellular milieu of cytoplasmic signalling molecules possibly interacting with the CRIT cytoplasmic tail is likely to differ between the species. In summary, the available data, which include both CRIT molecules being receptors for an identical ligand, nucleotide identity (including similarity of genomic organization), amino acid identity (and closeness of phylogenetic relationship) and secondary structure identity, point toward the principally haemopoietic, expressed human *CRIT* and *Schistosoma CRIT* genes being orthologous. That the CRIT receptor shares no significant overall sequence homology with other receptors—or, indeed, any other proteins in the database—and that there is significant sequence homology between *Schistosoma* and human CRIT suggest that *Schistosoma* CRIT arose as a result of a rare host-to-parasite HGT. A recently described example of another orthologous gene in *Schistosoma* and human, also

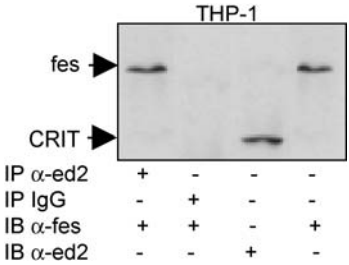

Fig. 4 CRIT associates with the cytoplasmic tyrosine kinase fes. Immunoprecipitation with the anti-CRIT-ed2 of Jurkat (T lymphocyte) and THP-1 (monocyte) cells and immunoblotting with anti-fes show CRIT to be associated with the cytoplasmic tyrosine kinase fes in THP-1 cells. No such association was shown in Jurkat cells, which possess CRIT but lack the fes tyrosine kinase (not shown)

presumed to be transferred from the host to the parasite through the intervention of mobile genetic elements, is that of the *S. mansoni* aspartic protease gene *cathepsin D* [28]. In schistosomes, this digests host haemoglobin from ingested human blood [1]. Although human lysosomal *cathepsin D* appears to also digest haemoglobin, in this case, its action is part of the recycling of old erythrocytes, which occurs in the liver and spleen.

Considering host complement regulators, which could disguise the parasite as 'host' in terms of the immune system, molecular mimicry in the parasite–host relationship can be achieved in three ways. The first mechanism involves directly adsorbing such proteins (e.g., human DAF) onto the schistosome surface [7], in this case from human erythrocytes. In the second mechanism, homologues of human proteins, such as DAF and CD59 are normally expressed by trypanosomes [22, 30] and schistosomes [33], respectively. The third mechanism, reviewed by Damian [3], involves naturally occurring retroviral vectors, which, having captured the host gene, can mediate HGT on infection of the parasite. Retroviral-related sequences were first found in schistosomes in preliminary studies in which antibodies against BALB virus 2 envelope glycoprotein (gp70) reacted with *Schistosoma* adult worms and in which homologous DNA sequences to the gag and pol regions of the ecotropic murine leukaemia virus were detected in Southern blots of *Schistosoma* adult worm DNA [39]. These rather tenuous findings were supported by later works in which in situ hybridization was used to show the transcription of host (mouse)-related DNA sequences in the adult worm

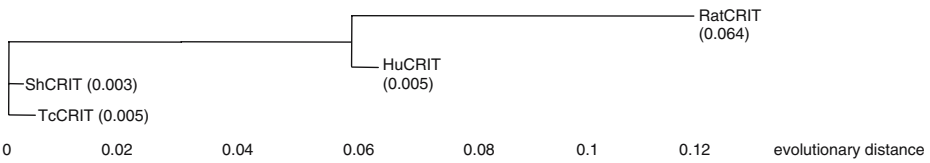

Fig. 5 Unrooted phylogenetic tree based on nucleotides 1–846 of the human CRIT. These were compared with the nucleotides of rat CRIT and those from the human parasites *S. haematobium* and *T. cruzi*. The topology algorithm used was that of the European Molecular Biology Laboratory, European Bioinformatics Institute Molecular Biology Server. The evolutionary distance is represented by the *length of the line segments*, which is indicated in *brackets*

and infectious cercarial stages of *S. japonicum* and *S. mansoni*. Using ^{32}P-labelled probes of mouse type C and type A retroviruses and of the env-specific region of the mouse ecotropic type C retrovirus, hybridization was observed in subtegumental and inner tissues of *S. japonicum* and *S. mansoni* adults, respectively, but interestingly not in cercariae of either species [21]. In later confirmatory studies, it was found that mouse type A and type C retroviral sequences could be transmitted horizontally from the host to the schistosomes [12]. Others have also suggested the acquisition of host genes by parasites [36]; such genetic exchanges could explain the high sequence homology between the parasite (particularly *Schistosoma*) and the host. In other supportive works using in situ polymerase chain reaction (PCR) and hybridization, the histocompatibility complex (major histocompatibility complex or MHC) class I sequence of the mouse was found in the genome of *S. mansoni* [12], indicating the horizontal transmission of class I MHC from the host to the parasite [13]. Using similar techniques, the mouse type 2 Alu sequence (B2), a repetitive DNA sequence in the mouse genome, was found in the adult worm body of *S. mansoni* and *S. japonicum* as were mouse retrovirus-related sequences.

The best support for horizontal gene transmission between the host and the parasite would be to find a mobile genetic element; to this aim, other workers more recently identified a retrovirus-like LTR retrotransposon, called *Boudicca* [2]. Retrotransposons are mobile genetic elements that transpose by reverse transcription. They may be subdivided into LTR (long terminal repeats) retrotransposons, which include retroviruses, and non-LTR retrotransposons or long interspersed nucleotidic elements, which lack LTRs. Some LTR transposons have been misclassified as transposable elements, which, having been subsequently found to be infectious, should be reclassified as endogenous retroviruses. Similarly, it has been speculated for some time that today's retrotranposons originated from ancient endogenous retroviruses that lost their infectivity. Approximately 20% of the *Schistosoma* genome of about 270 Mbp [37] is believed to be made up of retrotransposons [25]. Recently, DeMarco et al. [5] described four new ones: Saci-1, Saci-2 and Saci-3, which are of the LTR variety, and one non-LTR-expressed retrotransposon Perere, which is integrated into the *S. mansoni* genome. Characterization of the retrotransposon *Boudicca* revealed three likely ORFs with 5′ and 3′ LTRs of 328 bp. ORF1 consists of a retrovirus-like major homology region and a Cys/His box motif, as is typically found in the Gag polyprotein of similar retrotransposons and retroviruses. ORF2 contains contiguous aspartic protease, reverse transcriptase, RNAase H and integrase enzymatic domains. This is a structure similar to that of a retrovirus polyprotein, as might be found in the gypsy/Ty3 retrotransposons; indeed, when the reverse transcriptase sequence of ORF2 was used to search the database, *Boudicca* was confirmed as a gypsy-like retrotransposon and shown to be closely related to CsRn1 from the oriental liver fluke *Clonorchis sinensis* and to kabuki from *Bombyx mori*. The third ORF at the 3′ end was deemed to encode an envelope protein, which, if confirmed, would confer the ability of host cell entry, enabling *Boudicca* to undergo vertical as well as horizontal transmission. This study also found over a thousand copies of *Boudicca* to be dispersed throughout the *Schistosoma* genome, which accounted for up to 4% of the genome of *S. mansoni*. The active transcription of *Boudicca* was confirmed in the sporocyst, cercaria and adult worm stages of *S. mansoni* by reverse transcription PCR.

CRIT phylogeny and parasite tropism for blood cells: implications for transmission of CRIT gene from host to parasite

A phylogenetic analysis at the nucleotide level of the sequences of human and rat CRIT and the parasite *S. haematobium* and *Trypanosoma cruzi* (Fig. 5) shows that the parasite cluster is no less related than rat CRIT is to human CRIT, implying that the most likely origin of parasite (*Schistosoma* or *Trypanosoma*) CRIT is indeed the host. Although host-to-parasite HGT has not been reported much, one example is that of the protease inhibitor chagasin of *T. cruzi* [35] another one occurring in the semiparasitic mite *Proctolaelaps regalis* of the fruit fly *Drosophila* [10]. As parasite genomes begin to be deciphered, more parasite homologues of host proteins are discovered; indeed, the latest transcriptome analysis of *S. japonicum* [11] and *S. mansoni* [40] parasites has revealed many receptors for growth factors, hormones and cytokines shared with humans.

The *CRIT* gene in *Schistosoma* is presumably of human origin. Despite being apparently intronless, although we cannot as yet rule out possible intron(s) in the 5′ and/or 3′ untranslated regions, CRIT is, like many G-protein-coupled receptors, not a pseudogene. As verification of the functional status of the transferred *CRIT* gene, we can confirm the expression status of the *Schistosoma CRIT* gene by the presence of an expressed sequence tag (EST) for CRIT in the *Schistosoma* EST database. This replaces the need for confirmatory expression data using other empirical methods, such as primer extension analysis. That CRIT is expressed as a functional protein [14] and that it is therefore not a pseudogene are further shown by the recognition of the native 32-kDa *Schistosoma* protein with anti-CRIT-ed1 in a lysate of the adult worm. In other experiments, *Schistosoma* CRIT ORF was expressed as a recombinant protein recognized by the vaccination serum VBabS [14]. Human CRIT was expressed using a cell-free in vitro transcription/translation system; here, a ~32-kDa protein was recognized by anti-CRIT-ed1, anti-ed2 and anti-id2 [16].

As well as in *Schistosoma*, the *CRIT* gene is found in the kinetoplastid parasite *T. cruzi* [14, 16]. The trypomastigote stage of this parasite invades cells (typically of the heart muscle or liver) and white blood cells, and reproduces as amastigote. On cell death, some amastigotes may infect new cells, but others will transform into trypomastigotes, remaining in the bloodstream until the vector takes another blood meal. With the presence of *T. cruzi* trypomastigotes in peripheral blood, it is clear why they need to express CRIT. This may be found to be even more apparent in the related *T. brucei*, the causative agent of African sleeping sickness, because here the trypomastigotes replicate asexually solely in the peripheral blood. On these grounds, we can also understand the need for CRIT expression on the schistosome larval stage, especially the adult worm, which may live for up to 5 years in the host vasculature, as well as the released eggs.

Further evidence that the *Schistosoma CRIT* gene and probably that of *T. cruzi* were acquired independently via HGT is the lack of ubiquity amongst human parasites living in the vasculature. For example, CRIT is not found in *Plasmodium falciparum*. We were unable to find a CRIT homologue of this parasite by Western blot analysis using anti-ed1 antibody, or by PCR using degenerate oligonucleotides (unpublished data). More recently, this has been confirmed by similarity searches of the virtually completely sequenced *P. falciparum* genome. *P. falciparum* parasites are also believed to acquire host genes by HGT; in the case of CRIT, it is interesting to speculate that its absence in this parasite may be because *P. falciparum* spends a large part of its life cycle replicating as a merozoite in the protected environment of the host's erythrocytes, which, curiously, do not express CRIT [16]. CRIT is

also absent from *Leishmania major*, another protozoan of the order Kinetoplastida. This parasite reproduces as an amastigote within circulating macrophages, which also lack CRIT expression [16]. For now, whether CRIT is absent from parasites that spend only a limited part of their life cycle in the peripheral blood of the host, or is absent due to a lack of mobile genetic elements, or is absent because the *CRIT* gene has been lost from certain parasites, remains open to speculation.

Hitherto, gene transfers amongst eukaryotes were thought to occur rarely, although this perception is changing. The plethora of new eukaryotic genome sequences becoming available is only now providing material for phylogenomic analysis. Whilst HGTs are relatively rare amongst multicellular eukaryotes, this is not the case with regard to unicellular eukaryotes, such as *Trypanosoma* [9], which lacks a separate germ line and thus must tolerate the acquisition of foreign genes to survive.

CRIT is found in the teleost cod fish *Gadhus morhua*

The *CRIT* gene was found in cod ([16] and further work to be reported elsewhere), suggesting that *CRIT* genes may have evolved from a common ancestral gene, at least dating as far back as the earliest teleosts. CRIT is a receptor for mammalian C2; but in teleost fish, in particular in cod, although there is a classical pathway [27], there appears to be functional redundancy between alternative and classical pathways and, recently, fB/C2 genes have been isolated from carp [29], zebrafish [8] and medaka fish [24]. In evolutionary terms, the teleosts show the first compartmentalization of the immune system, including the first appearance of an important lymphoid organ—the thymus. Within the thymus, CRIT appears to be expressed in some thymocytes and macrophages, and possibly in Hassall's bodies as determined by immunohistochemistry using anti-CRIT-ed1 antibody and by in situ hybridization using an antisense CRIT probe (Lange, S., unpublished data). As in humans, by immunoblotting, we found CRIT to be present on cod lymphocytes. Further work will be needed to establish whether CRIT has a complement regulatory or as yet unidentified role in fish.

Conclusions

CRIT is a complement regulatory receptor in humans, which, in either its monomeric or dimeric form, attempts to regulate the amount of complement activation on the autologous cell surface by competing with C4b for binding C2. Furthermore, C2 bound to CRIT cannot be activated by C1s cleavage, so a 'variant' C3 convertase cannot be formed. CRIT is also found in the *Schistosoma* parasite, located in blood-filled pits and channels within the surface tegument of the adult worm. CRIT is also found in *T. cruzi*, although it has not been localized and we suppose from a phylogenetic analysis at the nucleotide level that the *CRIT* gene was acquired by these human parasites by the so far rarely described process of HGT from the host. Interestingly, CRIT is not found in the free-living nematode worm *C. elegans* and appears not to exist in *Drosophila melanogaster*. In both *Schistosoma* and humans, CRIT is phosphorylated on tyrosine, with the molecule having nine tyrosine residues in its cytoplasmic tail and interacting with the cytoplasmic tyrosine kinase fes, which is possibly associated with terminal myeloid cell differentiation. In evolutionary terms, CRIT is found at

least as far back as the early teleosts, having been cloned from the cod fish; this begs the question as to whether it is also found in the more primitive echinoderms. Looking at the CRIT family as a whole, besides the described function in complement regulation, we believe that there are alternative functions relating to the receptor's signalling capacity within the cell. It will be interesting to study these in relation to the various CRIT species along the phylogenetic tree.

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