COMPLEMENT EVASION BY PARASITES: SEARCH FOR "ACHILLES' HEEL"

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PREFACE

Parasitic diseases continue to be a major cause of human morbidity and mortality. Each year, more than one billion people are affected by one or more of the common human parasites and more than one million infected patients die. Generally, mammalian parasites are divided into protozoan parasites which include the *Trypanosoma*, *Leishmania*, *Entamoeba*, *Giardia*, *Plasmodium*, *Babesia* and *Toxoplasma* and to helminthic parasites which can be subdivided into trematodes (e.g. *Schistosoma* and *Fasciola*), cestodes (e.g. *Taenia* and *Echinococcus*) and nematodes (e.g. *Trichinella* and *Onchocerca*). The parasites contain multiple antigenic determinants which are recognized by the host's immune system as non-self and may potentially trigger an immune response that will destroy the parasite. This has been proven by serological and skin tests. Nevertheless, parasites are equipped with multiple, sophisticated immune evasion mechanisms which permit them to be infective and pathogenic (1-8). Intensive efforts in the past decade to produce a human parasite vaccine have not been successful yet and perhaps, as recently suggested (9) this issue will have to be reconsidered and efforts reoriented. This summary will focus primarily on the interaction of parasites with the complement system. Recent progress made in the elucidation of the complement evasion mechanisms of certain parasites will be discussed in light of the urgent need to develop new strategies for immunising against parasites.

COMPLEMENT AT LARGE

The complement system is one of the weapons the body utilize to fight intruders like bacteria, viruses and parasites. To achieve maximal protection, the complement system enlists several effector mechanisms. On one hand, it directly attacks the surface of pathogenic agents via the membranolytic attack complex, C5b-9 (MAC), and on the other hand it can induce and potentiate opsonization, chemotaxis and many other inflammatory processes which culminate in lysis and clearance of the intruder. More than 30 different proteins participate in this concentrated effort of the complement system to eliminate potential pathogens. Twenty of them are soluble plasma proteins and the others are membrane proteins acting as receptors or regulatory molecules. Several detailed reviews and books have been recently published which describe the complement proteins and genes and their molecular organization and functions (e.g. 10-16).

The complement cascade is activated via the classical or alternative pathway, mostly but not exclusively, by antibodies or complex carbohydrates, respectively. Each pathway employs a distinct set of proteins to produce proteases which cleave the C3 and C5 complement components. These are called the C3 and C5 convertases. Despite the fact that the convertases of the classical and alternative pathways are composed of distinct proteins, they cleave identical sites in C3 and C5. Cleavage of the C3 molecule by the C3 convertases (and by trypsin) sensitizes a metastable intra-molecular thioester bond and facilitates covalent binding of the resulting C3b fragment, by an ester or amide bond, to receptive surface molecules. This step of opsonization by C3b molecules is down-regulated by the combined action of the complement protease Factor I with Factor H or with the membrane proteins Complement receptor type 1 (CR1, CD35), CR2 (CD21) or membrane cofactor protein (MCP, CD46). The resulting inactive C3b (iC3b) serves as a ligand to two other complement receptors, CR3 (CD11b,CD18) and CR4 (CD11c,CD18) which are present on various leukocytes (11,16). Binding via these receptors amplifies phagocytosis and secretion of various inflammatory agents (10,11,15-17).

Cleavage of C5 by the C5 convertases serves as the trigger for the assembly of the MAC. C5b, C6, C7 and C8 associate together to bind multiple C9 molecules and induce their polymerization into a cylinder-like structure which produce, by a yet unknown mechanism, cell lysis (14,18). Furthermore, under certain conditions, MAC assembly and cell lysis may proceed in the absence of classical or alternative complement activation. This phenomenon, called `reactive lysis' (19) was recently described in *Entamoeba histolytica* trophozoites which are lysed by complement following fluid phase cleavage of C3 by a 56 kDa parasitic cysteinyl protease (20).

COMPLEMENT AND PARASITES: SELECTION OF THE FIT

Most parasites studied so far have been shown to contain membrane bound, secreted or extractable substances which activate *in vitro* complement via the classical or alternative pathway or both. *In vivo* studies in infected patients or animals have provided further indication for the capacity of these parasites to induce complement activation in naive and immunized hosts. Certain parasites (e.g. *Leishmania*, *Trypanosoma* and *Toxoplasma*) take advantage of the C1q, C3b and iC3b molecules deposited on their surface to bind to complement receptors on their host cells, facilitate their entry and protect themselves against cytotoxic substances produced by these cells. This subject has been reviewed recently (21,22) and will not be discussed here. Due to space limit, this summary will describe and discuss only selected studies out of the many performed on the interaction of parasites with complement. Further details may be found in one of the comprehensive review articles published recently (7,21-25).

As the title of this chapter implies, it is the complement resistant parasites or parasite stages which are virulent and successfully survive within their host. Thus, pathogenic strains of *E. histolytica* isolated from patients with colitis and amebic liver abscess are resistant to complement-mediated killing, whereas isolates from asymptomatic patients are readily lyzed by non immune serum (26). Culture of complement-sensitive, non-infective *E. histolytica* trophozoites *in vitro* with human (27) or bovine (28) serum results in appearance of complement-resistant amoeba. Whether the result of selection or adaptation, the complement-resistant amoeba are highly infective in hamsters and produce multiple liver abcesses (28). Interestingly, both complement-resistant and -sensitive amoeba activate complement efficiently in the fluid phase (27,28). Similarly, unlike non pathogenic amoeba of *Naegleria fowleri*, the pathogenic amoeba are resistant to complement-mediated lysis (29).

Resistance to complement-mediated lysis is displayed by the trypomastigote forms of *Trypanosoma cruzi*, the parasite causing Chagas disease in tropical America (30,31) and *T. brucei* the cause of sleeping sickness in Africa (32). Epimastigotes, which are the noninfective forms multiplying in the gut of the insect vector are killed efficiently by complement. In contrast, the infective trypomastigote forms found in the hindgut and feces of the insect vector or in the blood stream of infected animals are complement resistant (30). Once they enter their host cell, the trypomastigotes transform into amastigotes which multiply and transform again into trypomastigotes. The cycle is continued by release of the trypomastigotes and amastigotes which infect new cells (33). The amastigote form is also resistant to complement lysis (34). Despite the distinct susceptibility to complement of the epimastigote, trypomastigote and amastigote forms of *T cruzi* they all activate the complement cascade. The basis for the complement resistance of the trypomastigote and amastigote forms will be described in the next chapter.

Infection by *Leishmania* spp is produced by a sandfly vector which transmits into the host skin the promastigote form of the parasite. The latter form infects macrophages, multiplies and transforms into amastigotes which are released and infect other cells. Amastigotes of the various *Leishmania* spp are sensitive to lysis by human complement, though amastigotes of *L. donovani*, the parasite causing visceral Leishmaniasis, appear to be more resistance than the others (35). The promastigotes of *Leishmania* show distinct susceptibility to complement-mediated lysis depending on their growth phase (reviewed in 21,22). Promastigotes grown *in vitro* to logarithmic stage (LOG) are sensitive and those grown to stationary stage (MP, metacyclic promastigotes) are resistant to complement (36,37). Interestingly, the LOG promastigotes are noninfective whereas the MP's are highly infective for animals and cells. These tissue culture LOGs and MPs probably represent developmentally regulated stages of promastigotes present in the gut of the infected sandfly vector (38).

The parasitic worm *Schistosoma mansoni* also undergoes a stage-specific transformation which permits it to resist complement-mediated damage (reviewed in 7,23,25). The free-swimming cercaria shed by the snail vector activates complement very efficiently (39). However, the schistosomula derived after skin penetration and the more mature forms, the lung worms and the adult worms found in the host's mesenteric veins 6 weeks after infection, are much less sensitive to lysis by complement (40-43). By applying in vitro mechanical force to cercariae, they lose their tails and undergo a series of transformational events which resemble the in vivo events (reviewed in 43). Relevant to this discussion, they rapidly convert from a complement-sensitive to complement-resistant form (25,43-45). The basis for this conversion will be discussed in the next chapter.

COMPLEMENT RESISTANCE: `PASSIVE' AND `ACTIVE' MECHANISMS

The strategies utilized by parasites to resist complement-mediated lysis can be generally classified into `passive' and `active' strategies. `Passive' evasion mechanisms rely on the absence of antigenic epitopes or complement activating substances on the parasite's surface. This is achieved in many parasites by a process known as `Molecular Mimicry', i.e. the sharing of antigenic determinants between parasite and host (46,47). The parasite is thus recognized by the host as `self'. Schistosomula of *S. mansoni* which transform in the host's skin from a complement sensitive state to a complement resistant state, achieve this goal by rapidly shedding from their surface the glycocalyx coat which is a strong direct activator of the alternative pathway of complement and is also immunogenic (25,45,48,49). Several host serum proteins (including MHC antigens, fibronectin, IgG, C1q and 2 macroglobulin) were found on the surface of the transformed larvae and the more mature worms (46,47). `Hiding behind' a surface coat which is impermeable to complement, like the peptidoglycan cell wall of gram-positive bacteria (50), may also be regarded as a passive mechanism of resistance. The schistosomular body is surrounded by a unique double bilayer membrane which may similarly interfere with the cytolytic action of complement (43).

`Active' resistance is obtained by mechanisms which actively interfere with the lytic action of complement. These include specific inhibition of the complement cascade, proteolytic degradation of complement proteins bound to the parasite, release of the bound complement proteins and activation of the complement cascade in the fluid phase (i.e. complement consumption). Evidently, parasites were familiar with these options and utilized them to their benefit. The complement resistant trypomastigotes of *T. cruzi* produce a complement inhibitor(s) which interfere with the formation and stability of the C3 convertase. As described in three independent reports, this inhibitory activity is expressed by a secreted protein of 60 kDa (51) or 87-93 kDa (52) or a membrane protein of 160 kDa (53) molecular mass. Antibodies against the 87-93 kDa and 160 kDa molecules which block their inhibitory activity, sensitize the trypomastigotes to complement-mediated lysis. It is not clear yet whether these proteins are related to each other, however the 87-93 kDa and 160 kDa appear to show sequence homology (22,54) to human DAF (decay accelerating factor, CD55) which is a membrane protein restricting C3 convertase activity on human blood, epithelial and endothelial cells (reviewed in 11). Gp 58/68 is another protein expressed on the surface of trypomastigotes of *T. cruzi* which inhibits formation of the alternative pathway C3 convertase (55). This molecule which acts also as a fibronectin/collagen receptor, blocks formation of the convertase by a mechanism different from that of DAF and Factor H, which has not yet been defined. Enzymatic treatment of the trypomastigotes with trypsin and pepsin but also with neuraminidase and glycanase (56) promoted C3 deposition and lysis of the metacyclic trypomastigotes. This may suggest the presence of surface sialic acid residues which potentiate the regulatory action of Factors H and I on deposited C3b (57,58). In fact, it has been reported that Factor H binds with higher affinity to C3b deposited on metacyclic trypomastigotes than to epimastigotes (59). A completely different resistance mechanism is probably operative in the amastigote form of *T. cruzi*. Similar amounts of C3 and C5b-7 are deposited on amastigotes and epimastigotes (34) . Yet, 4-6 times less C9 bind to amastigotes and the bound and/or polymerized C9 could be removed from the amastigotes surface by trypsin treatment. It was suggested (34) that a pronase-sensitive surface protein inhibits insertion of the C5b-9 complex into the amastigotes membrane.

Different strategies of complement evasion have been described in promastigotes of *L. donovani* and of *L. major*. The *L. donovani* promastigotes block the complement activation at the C3 step. They cleave rapidly the deposited C3b to iC3b and smaller fragments and release the bound C3b from its acceptor molecules (37). The mechanism has not been resolved yet, though it has been suggested that gp63, the surface protease of *Leishmania* plays a role in the proteolytic degradation of bound iC3b (22,60). In *L. major* infective promastigotes, effective down regulation of the complement cascade occurs at the MAC binding step. Apparently, C3b and C5b-7 are similarly deposited on complement-resistant and sensitive promastigotes, however the C5b-9 complex fails to insert properly and is released from the surface membrane of the complement resistant form (60). A candidate inhibitor of C5b-9 membrane insertion is the lipophosphoglycan which is abundant on the surface of *Leishmania* (22).

We have recently proposed that processes of protein phosphorylation may serve to protect human leukemic cells against complement-mediated lysis (61) *Leishmania* promastigotes probably use a similar mechanism. Both *L. donovani* and *L. major* promastigotes possess a cell surface ecto-protein kinase activity (62,63). As recently demonstrated, the *L. major* ecto-kinase(s) can phosphorylate C3, C3b, C3a, C5 and C9 (64). It still remains to be determined whether phosphorylation of complement components

increases the infectivity of *Leishmania* by protecting it against complement and/or promoting parasite penetration into macrophages via C3 receptors.

The Complement resistant stages of *S. mansoni* are equipped with several escape mechanisms which probably act concomitantly to ensure safe blockage of the cytolytic action of complement and of complement-activated effector cells like eosinophils, neutrophils and monocytes. As already mentioned, one of the first steps the infecting parasite takes is shedding of the glycocalyx coat which has protected it against osmotic shock in fresh water. By doing this, the worm becomes a poor activator of the alternative complement pathway and less immunogenic (25,45,48,49). Glycocalyx release is accelerated by proteolysis by a 28 kDa serine protease secreted from glands within the cercaria (25,65-67). Since the released glycocalyx is such a strong complement activator via the alternative pathway (45,49), it may consume complement thus reducing the effective complement concentration around the penetrating larvae. This claim is supported by the finding that dilution of normal human serum from 90 to 50% reduces the killing of complement sensitive schistosomula from 100 to 63% and prolonges the time of maximal killing from 2 hrs to 22 hrs (45). Similar shedding of glycocalyx has been reported in newly excysted juveniles of *Fasciola hepatica* (68). Two types of results indicated that the glycocalyx-free schistosomula of *S. mansoni* are not just `passively' protected from complement recognition and killing: 1. Polyclonal rabbit antibodies bound to chemically-modified antigens (TNPylated or DNPylated) on the surface of the schistosomula failed to induce efficient complement-mediated parasite killing (69,70), and 2. Treatment of the schistosomula with trypsin and pronase sensitized the schistosomula to lysis by the alternative complement pathway (71). Recent results (72,73 and unpublished) indicated the presence of two distinct complement inhibitors on the surface of both schistosomula and adult worms of *S. mansoni*. One inhibitor blocks the complement cascade at the C3 step by a mechanism not fully clear yet. Even after extraction from the parasites with detergent or trypsin, this inhibitor can prevent C3b deposition on rabbit erythrocytes and antibody-sensitized sheep erythrocytes. The possibility that this inhibition is exerted by a 70 kDa C3b-binding membrane protein is now being examined. The second complement inhibitor prevents MAC formation. Schistosomula and adult worms of S. mansoni contain on their surface a CD59-(P-18)-like molecule (74). This molecule has been found on human erythrocytes and leukocytes (Mr = 18-21 kDa) (75,76), and on platelets (Mr = 37 kDa) (77). CD59 blocks MAC formation on human cells probably by inhibiting interaction of C9 with C5b-8 (78). The molecular size of the schistosomular CD59-like molecule is still under investigation. Antibodies directed against human CD59 bind to schistosomula and induce their killing via the alternative pathway of complement (74). The latter two complement inhibitors are intrinsic molecules produced by a parasitic larvae which had no previous contact with host's tissue. However, schistosomula of *S. mansoni* have also the capacity to absorb to their surface complement inhibitors from host's tissues. Thus, DAF was transferred in vitro from human erythrocytes to schistosomula (79) and DAF was also detected on the surface of worms recovered from different infected animals (80). The mechanism underlying this molecular "migration" still awaits clarification. Yet, another protective mechanism probably employed by schistosomula of *S. mansoni* is the proteolytic degradation of complement proteins such as C3, C3b, iC3b and C9 by a membrane ecto-protease (72,73). This is a 28 kDa serine protease which appears to be related or identical to the cercarial secreted protease (65,71). It cleaves very efficiently human iC3b at two sites: between Glu 967 and Met 968 in the 1 chain and between Tyr 1326 and His 1327 in the 2 chain (Ghendler, Arnon and Fishelson, unpublished). On the basis of these findings, we have suggested that schistosomula are thus protected against iC3b-mediated effector cell killing. Indeed, *in vitro* treatment of schistosomula with Soybean trypsin inhibitor or phenylmethylsulfonyl fluoride prior to complement activation markedly potentiated the schistosomacidal activity of human neutrophils (73).

CONCLUDING REMARKS

Invading organisms, including parasites, would be expected to be destroyed by the effector arms of the immune system, i.e. complement and cytotoxic cells. Yet, by utilizing sophisticated mechanisms of immune evasion and resistance, the virulent parasites can successfully infect their hosts and persistently reside in them. In particular, complement resistance appears to be an important virulence factor. Major progress has been made in the past years in the elucidation of the evasion mechanisms in *T. cruzi*, *L. major*, *L. donovani* and *S. mansoni* on the molecular level. However, this field is rapidly expanding now and additional complement evasion molecules will probably be soon described. The physiological significance of each of these escape mechanisms has to be evaluated in different experimental models. Most importantly, such experiments may disclose to us the sensitive *Achilles' heel* of each of the parasites, i.e. the molecules against which the host immune system should be `encouraged' to react. To date, many attempts to produce human parasite vaccines have not been successful. This may be due to the fact that

the main factor considered so far was the immunogenicity of the proposed vaccine molecule. Production of blocking antibodies against vital surface parasitic molecules such as complement inhibitors or ecto-enzymes may prove to be more successful. This idea is strongly supported by the findings that antibodies to the 160 kDa protein of *T. cruzi* (53) or the CD59-like molecule of *S mansoni* (74) induced efficient *in vitro* parasite killing and that antibodies against the major glycolipid of *L. major* reduced parasitemia in mice (81). Furthermore, nature has proven to us that immunity against parasites can be achieved. 'Concomitant immunity' is a state of resistance to reinfection in hosts which are already infected (82). Whether the result of a shared antigen between resident and infective forms or of a stage-specific antigen (9), apparently, resident parasites have succeeded where we have so far failed.

Acknowledgements

The author's research described in this review was supported by the MacArthur Foundation and the UNDP/World Bank/WHO. The author is grateful to Dr Ken Reid for critically reading this review.

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