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Sequestration of host-CD59 as potential immune evasion strategy of *Trichomonas vaginalis*

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ABSTRACT

Trichomonas vaginalis is known to evade complement-mediated lysis. Because the genome of *T. vaginalis* does not possess DNA sequence with homology to human protectin (CD59), a complement lysis restricting factor, we tested the hypothesis that host CD59 acquisition by *T. vaginalis* organisms mediates resistance to complement killing. This hypothesis was based on the fact that trichomonads are known to associate with host proteins. No CD59 was detected on the surface of *T. vaginalis* grown in serum-based medium using as probe anti-CD59 monoclonal antibody (MAb). We, therefore, infected mice intraperitoneally with live *T. vaginalis*, and trichomonads harvested from ascites were tested for binding of CD59. Immunofluorescence showed that parasites had surface CD59. Furthermore, as mouse erythrocytes (RBCs) possess membrane-associated CD59, and trichomonads use RBCs as a nutrient source, organisms were co-cultured with murine RBCs for one week. Parasites were shown to have detectable surface CD59. Importantly, live *T. vaginalis* with bound CD59 were compared with batch-grown parasites without surface-associated CD59 for sensitivity to complement in human serum. Trichomonads without surface-bound CD59 had a higher level of killing by complement than did parasites with surface CD59. These data show that host CD59 acquired onto the surface by live *T. vaginalis* may be an alternative mechanism for complement evasion. We describe a novel strategy by *T. vaginalis* consistent with host protein procurement by this parasite to evade the lytic action of complement.

1. Introduction

It is well appreciated that *Trichomonas vaginalis* is the causative agent for a major sexually transmitted infection (STI) worldwide, and this STI causes significant adverse health outcomes for both women and men (Harp and Chowdhury, 2011; WHO, 2012). A great deal is known about the complex host-parasite interrelationship and the many aspects of host pathogenesis mediated by trichomonad virulence factors (Figueroa-Angulo et al., 2012). For example, some of the many proteases synthesized by *T. vaginalis* that are regulated by iron are known to be important in numerous aspects of the biology of the organism and are essential for host infection and survival (Alderete et al., 1992, 1995; Provenzano and Alderete, 1995; Figueroa-Angulo et al., 2012). It is known that trichomonads are sensitive to complement-mediated lysis, and high-iron-regulated cysteine proteases protect the organism from complement (Demes et al., 1988; Lehker and Alderete, 1992; Alderete et al., 1995; Figueroa-Angulo et al., 2012).

It is plausible for *T. vaginalis*, and perhaps other pathogens, to possess redundant mechanisms for immune evasion, such as for protection against complement. Thus, it is conceivable that trichomonads bind host factors important for resistance to complement. For example, membrane attack complex (MAC) lysis on autologous cells is aborted by the presence of the protein called protectin (CD59) (Meri et al., 1996; Zipfel and Skerka, 2009; Sarma and Ward, 2011). This protein is a glycosyl-phosphatidylinositol (GPI)-linked membrane protein expressed on diverse cells, including RBCs and endothelial and epithelial cells of the urogenital tract (Daniels, 1999; Farkas et al., 2002). It is known that CD59 prevents

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MAC formation by inhibiting both C9 complexing with C5b-C8 and the mechanism of pore formation (Farkas et al., 2002; Kimberley et al., 2007).

We hypothesized that, as *T. vaginalis* does not possess any DNA with nucleotide sequence homology to human CD59, surface acquisition of CD59 by *T. vaginalis* would lead to resistance to complement-mediated lysis in vivo. We based this hypothesis on several known facts. First, early on it was established that live *T. vaginalis* organisms were capable of associating with host serum proteins both loosely and avidly (Peterson and Alderete, 1982, 1984a,b,c). One special feature of host protein binding is related to nutrition, such as lipid uptake (Peterson and Alderete, 1984b,c) and iron procurement for regulating gene expression (Peterson and Alderete, 1984a; Lehker and Alderete, 1992). This ability of *T. vaginalis* and microbial pathogens to appropriate host proteins with special functions and in a specific receptor-mediated fashion now represents a theme among microorganisms that is important to the host–parasite interaction. Second, cytoadherence and cytotoxicity of host cells including lysis of RBCs by *T. vaginalis* represents another nutrient acquisition mechanism that permits growth and multiplication in medium in the absence of serum (Lehker et al., 1990; Lehker and Alderete, 1992). Third, an important feature of CD59 is its ability to transfer among the surfaces of neighboring cells (Kooyman et al., 1995). A consequence of contact by *T. vaginalis* with a cell possessing CD59 is that this may lead to transfer of CD59 to the parasite surface. Therefore, this testable hypothesis is the acquisition of CD59 from the host concomitant with enhanced resistance to complement-mediated lysis.

This paper describes the ability of *T. vaginalis* to acquire host CD59. Trichomonads obtained from ascites after infection of mice as well as parasites co-cultured with mice RBCs had surface-associated CD59. These organisms compared with batch-culture parasites without surface CD59 possessed a greater ability to resist complement lysis. This property of *T. vaginalis* procurement of CD59 from the host appears to be a novel, alternative mechanism of immune evasion that confers upon the parasite the ability to survive within the host environment. To our knowledge this is the first time that CD59 on *T. vaginalis*, and among other human parasites, has been reported.

2. Materials and methods

2.1. Absence of CD59 DNA sequence homology in the T. vaginalis genome

To determine whether *T. vaginalis* possessed any DNA sequence with homology to the known human CD59 gene sequence (GenBank database http://www.ncbi.nlm.nih.gov/GenBank/index.html, accession number CR407661), we performed BLAST analysis with the NCBI published sequence database of the *T. vaginalis* DNA (TrichDB, http:www.trichdb.gov and GenBank database, http:www.ncbi.nlm.nih.gov). The *T. vaginalis* DNA did not possess any sequence homology to the human gene sequence.

2.2. T. vaginalis organisms

T. vaginalis isolates C-1:NIH and IR 78 were obtained from the American Type Culture Collection and grown in TYM medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (Ibáñez Escribano et al., 2012). Trichomonads were batch cultured in glass tubes at 37 °C and passaged every 48–72 h.

For some experiments organisms were co-cultured with murine RBCs obtained by retro-orbital bleeding. One milliliter of blood with heparin was centrifuged at 350 × *g* for 5 min. RBCs were washed twice in PBS and resuspended in 0.5 ml of PBS and stored at 4 °C prior to use. The RBCs were examined for the presence of CD59 by immunofluorescence prior to use in co-culture experiments as described below. For co-cultures, passaged organisms were supplemented each time with RBC suspension (parasite:erythrocyte ratio 1:50). Trichomonads were in the presence of erythrocytes at least 7 days prior to use in fluorescence experiments. Co-cultured parasites used for immunofluorescence as described below had no detectable RBCs.

2.3. Infection of mice with T. vaginalis for ascites with trichomonads

For infection of female mice from the Naval Medical Research Institute, batch cultured *T. vaginalis* C-1:NIH isolate trichomonads were washed once in PBS and suspended in TYM medium without serum. Mice were then infected intraperitoneally with 15×10^6 parasites in a 0.6 ml volume. After 15 days post-infection, ~1 ml of intraperitoneal fluid was collected. The ascites was examined by brightfield microscopy, and 100% of visible parasites were highly motile. The organisms were then processed as indicated below for individual experiments. All animal welfare and experimental conditions were carried out according to the Directive 2010/63/EU of the European Parliament and the Council of the European Union and controlled in Spain by Royal Decree 53/2013 of 1 February on the protection of animals used for scientific purposes.

2.4. Antibodies to adhesin AP65 and CD59

For confocal studies, the hybridoma supernatant containing IgG_1 monoclonal antibody (MAb) 12G4 was used as a control for all experiments to detect the *T. vaginalis* AP65 adhesin on the trichomonal surface under these experimental conditions (Garcia et al., 2003). This surface detection by 12G4 permits determination of the surface reactivity with the MAb to CD59. Further, reactions of parasites derived from ascites as above confirm that the specific fluorescence detected by the MAb to CD59 is toward the trichomonads. The MAb 12G4 and the murine MAb to the mouse CD59 (Sigma–Aldrich Co., St. Louis, MO, USA) were diluted 1:200. Fluorescein isothiocyanate (FITC)-conjugated (diluted 1:200) and Alexa-conjugated anti-mouse IgG secondary antibodies (Sigma–Aldrich, Corp., St. Louis, MO, USA) (diluted 1:1.000) were used in the immunofluorescence assays for detection of AP65 and CD59, respectively, unless otherwise indicated.

2.5. Immunofluorescence to detect surface-associated AP65 and CD59

Live *T. vaginalis* organisms from batch cultures and isolated from mice ascites were washed twice in PBS. The sample was fixed o/n at 4 °C in a 2% formaldehyde solution. A volume containing ~50,000 fixed parasites were then washed twice in phosphate solution and added to individual acetone-resistant spots (Biomérieux, France). Samples were incubated with 100% acetone at 4 °C for 10 min and then treated with PBS containing 2% BSA for 1 h. After washing with PBS, the slides were incubated for 1 h at 37 °C with MAb diluted 1:200 in PBS. Slides were then washed twice with PBS containing 2% Tween 80 followed by incubation for 30 min with a 1:200 dilution of secondary FITC-conjugated rat anti-mouse IgG antibody (Sigma–Aldrich Chemical Corp., St. Louis, MO). Then, the slides were incubated for 30 min at 37 °C with a 1:200 dilution of MAb to CD59 (Sigma–Aldrich). Finally, after washing twice with PBS-2% Tween 80 to remove any excess Ab, slides were incubated for 30 min at 37 °C with 1:1000 dilution of secondary Alexa-conjugated IgG Ab. The samples were finally fixed with mounting liquid containing DAPI (Prolong® Gold antifade reagent with DAPI, Invitrogen, USA). After incubation at RT for 24 h, the trichomonads were examined

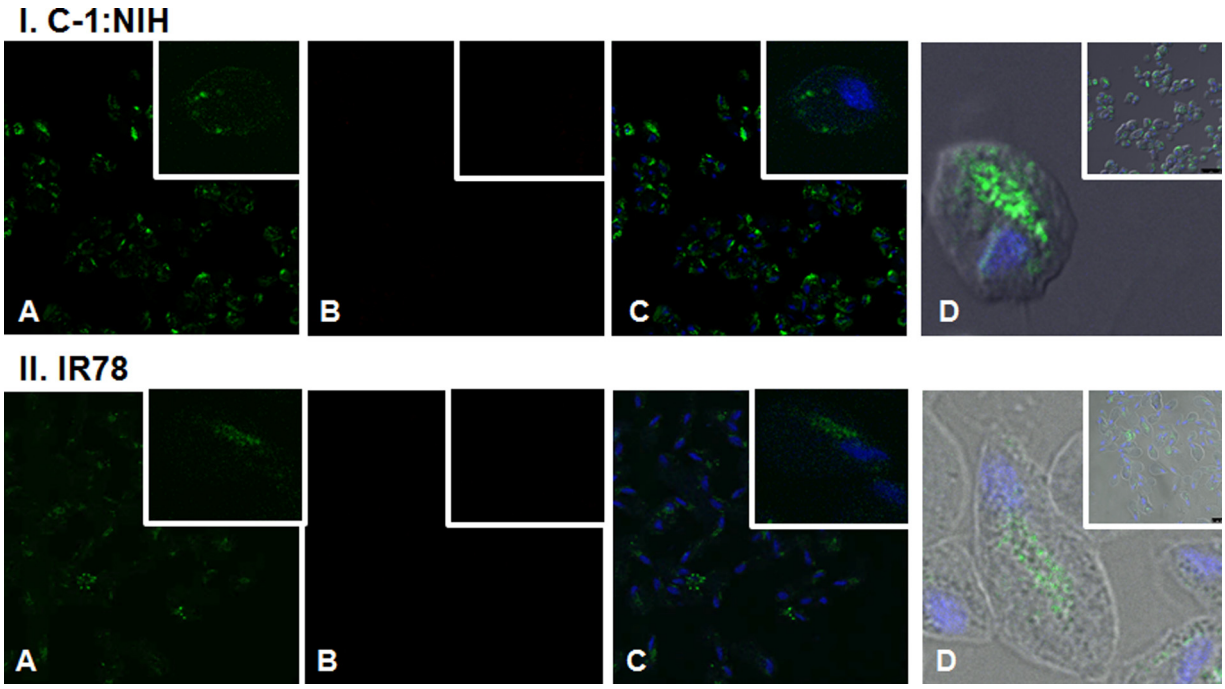

Fig. 1. Immunofluorescence of trichomonads of isolates C-1:NIH (part I) and IR 78 (part II) to show the presence of AP65 using as probe the 12G4 MAb (A) and the absence of CD59 (B) in batch-grown cultures of *T. vaginalis*. AP65 detection combined with DAPI staining (C) and an enlarged view of the parasite with the merged AP65 and DAPI staining with brightfield (D). The insets for panels A–C show an enlarged view of the parasite to illustrate in detail the immunofluorescent results. The size scales for parts I and II were 25 μm and 10 μm, respectively.

with a Leica TCS-SP5 confocal laser scanning microscope (Leica Microsystems, Madrid, Spain). These studies have been performed by the Confocal Microscopy Service of the Universidad de Alcalá de Henares (UAH) and the Biomedical Networking Center (CIBER-BBN), located at the facilities of the Cell Culture Unit (www.uah.es/enlaces/investigacion.shtm). The order of using MAb 12G4 to AP65 and secondary FITC-rat anti-mouse antibody followed by MAb to CD59 and Alexa-goat anti-mouse was important so as to avoid any cross-reactions among the secondary antibodies, as illustrated for Fig. 1. To further confirm the results of Fig. 1, fluorescence experiments were done individually with the primary antibodies but using only the FITC-anti-mouse secondary antibody, as can be seen in the insets for panels B and C of Fig. 2.

Because mice erythrocytes possess surface CD59 detectable with the MAb, we performed fluorescence experiments using mice RBC as positive controls throughout, as shown in Fig. 3. Absence of false positives was confirmed by using slides without trichomonads as negative controls that were treated with the same antibodies under identical conditions. Additional negative controls included duplicate slides containing parasites but that were treated only with the secondary antibodies. To insure absence of permeability and bona fide detection of proteins on the parasite surface we performed fluorescence using the IgG_1 MAb L64 that is reactive to a cytoplasmic protein of *T. vaginalis* (Alderete et al., 1987; Lama et al., 2009). All fluorescence experiments were reproducible and performed no less than three times using 2 replicates each time.

2.6. Determination of C3 in human serum

The concentration of C3 in human serum used for lytic assays to determine the extent of lysis by complement was determined by radial immunodiffusion assay (Easy RID, Liofilchem S.r.l.) as per the

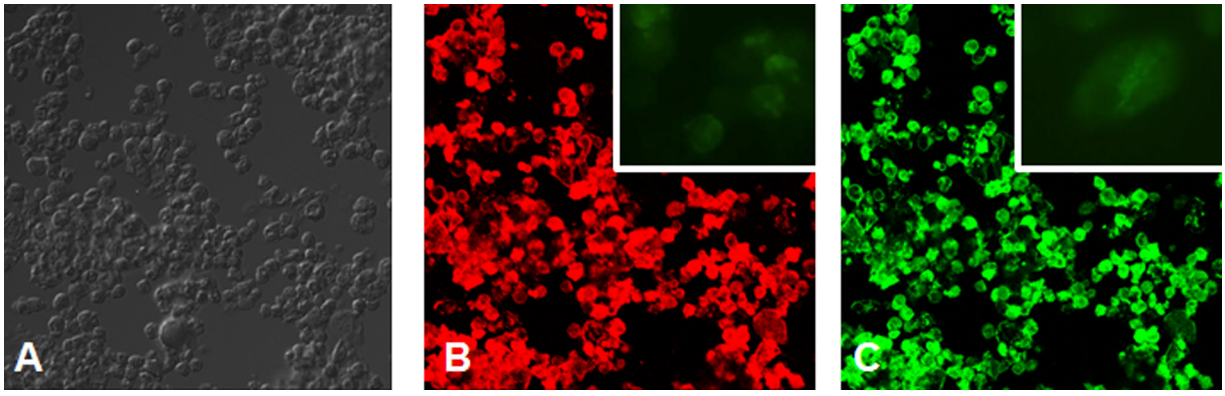

Fig. 2. Surface acquisition of CD59 by *T vaginalis* organisms derived from ascites after infection of mice with isolate C-1:NIH. Fluorescence experiments were performed using as probe the MAb to CD59 (B) and the MAb 12G4 to AP65 (C). The combined fluorescence for CD59 and AP65 (D), and the brightfield (A) are shown. Further, fluorescence experiments were done with either MAb to CD59 or MAb to AP65 but using only the FITC-anti-mouse secondary antibody. The insets for panels B and C show an enlarged image of the surface-associated CD59 and AP65 proteins. Negative controls were performed with absence of fluorescence as seen for panels B of Fig. 1. The size scale shown in panel A is 25 μm.

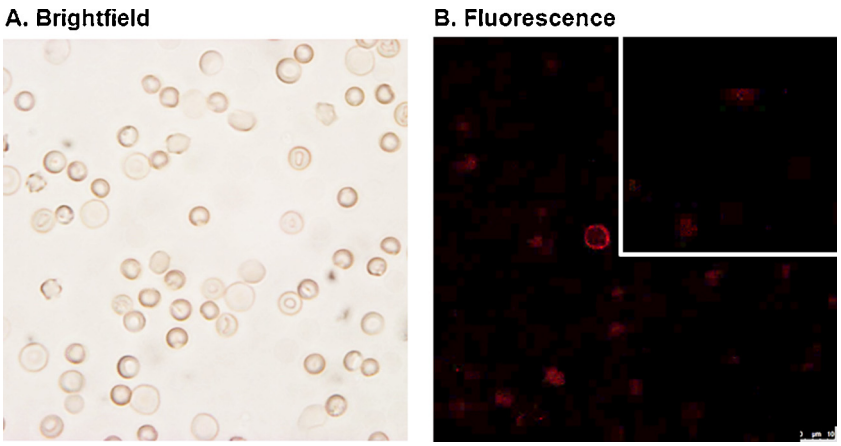

Fig. 3. Mice erythrocytes possess CD59 on the surface. Brightfield RBCs (A) were probed with MAb to CD59 (B). The size scale shown in panel A is 10 μm.

manufacturer's protocol with minor modifications. In our hands the incubation time for C3 determination was optimal at RT after 24 h in contrast to the recommended 48 h time period. Concentrations of C3 for our lysis assays ranged from 0.2 mg/ml to 1.2 mg/ml. A negative control in the absence of serum was included in all of the experiments.

2.7. Lytic assay to determine trichomonad susceptibility to complement lysis

Trichomonads of *T. vaginalis* isolates C-1:NIH and IR 78 grown in the presence and absence of mice RBCs as described above were washed once in PBS and suspended in fresh TYM-serum medium. Each of 7 tubes received 1 ml containing 2.5×10^5 live organisms as monitored by motility. To each tube was added 1 ml of different concentrations of complement. The different dilutions of serum were prepared in TYM-serum medium. The mixtures of organisms and human serum were then incubated at 37 °C for 1.5 h, a time that was optimal under these experimental conditions. We determined the lysis of trichomonads by a rapid fluorimetric method using resazurin as a redox dye, to determine the viability of remaining organisms. This redox dye is reduced to a fluorescent compound by reductases presented only in viable parasites. The determination of sensitivity to human sera is as described recently (Ibáñez Escribano et al., 2012). For all conditions, two independent experiments were performed using eight replicate samples. A standard curve was obtained as described before (Ibáñez Escribano et al., 2012) where a linear correlation was obtained. This fluorimetric assay permits excitation at the wavelength of 535 nm and an emission wavelength of 590 nm, which measures live organisms. Control samples without serum as well as without parasites were included in all experiments. The specific activities (sp act) defined as the amount of fluorescent emission measured for the control samples representing 100% of parasites were used in the calculations for normalizing the results and for presenting the data in Fig. 5. Each sp act was calculated from the average of the eight replicates.

3. Results

3.1. Batch culture T. vaginalis organisms do not have CD59 detectable by MAb to CD59

We wanted to perform experiments that show surface acquisition by trichomonads of CD59 from mice during intraperitoneal infection. We first confirmed the absence of any surface reactivity of batch-grown *T. vaginalis* organisms with the specific MAb reactive to murine CD59. Immunofluorescence was performed on trichomonads of two isolates using the MAbs as probes. As can be seen in Fig. 1 for isolates C-1:NIH (part I) and IR 78 (part II), the positive control MAb 12G4 to AP65 was used and readily detected green fluorescence with FITC-conjugated secondary antibody (panels A). In the same spot of the slide, we then incubated with MAb to CD59 (Section 2), and as can be seen, no red fluorescence with the secondary Alexa-conjugated anti-mouse IgG was detected (panels B). The integrity of trichomonads was evidenced by DAPI staining of DNA and combined with AP65 fluorescence (panels C) and the parasite morphology under brightfield with merged AP65 and DAPI staining (panels D). It is noteworthy that the use of MAb 12G4 and FITC-rat anti-mouse Ab first was important as we did not observe any non-specific fluorescence when we then used MAb to CD59 and Alexa-goat anti-mouse Ab. Further, individual experiments using only the MAb 12G4 to AP65 and of MAb to CD59 with the FITC-secondary Ab gave identical results (data not shown). The insets show an enlarged trichomonad obtained from another representative experiment to illustrate reproducibility and the nature of fluorescent detection of AP65 and DAPI as well as absent fluorescence with MAb to CD59. No fluorescence was ever observed using secondary antibody alone in all experiments. As an additional negative control, no fluorescence was detected using the MAb L64 to a trichomonad cytoplasmic protein of the same IgG_1 isotype as the 12G4 MAb (data not shown). Thus, this experiment indicates that trichomonads have no murine CD59-like protein on the surface.

3.2. Surface acquisition by trichomonads of murine CD59 during infection

We next recovered live *T. vaginalis* organisms from mice ascites after infection with isolate C-1:NIH trichomonads, and after washing, we performed immunofluorescence as above with the MAbs to CD59 and AP65. Fig. 2 shows that most, if not all, trichomonads have increased intensity of red fluorescence using the MAb to CD59 (panel B) and green fluorescence using the MAb 12G4 to AP65 (panel C). The trichomonads used for this fluorescence experiment showed excellent integrity (panel A). Importantly, to further confirm that the individual reactions to the AP65 and CD59 proteins with the respective FITC- and Alexa-secondary antibodies, we performed separate fluorescence experiments with the primary antibodies followed by FITC-anti-mouse secondary antibody. The insets for panels B and C show the detection by the respective MAbs and FITC-secondary Ab. Again, this is evidence of surface placement of both AP65 and CD59 proteins. The high intensity of fluorescence observed in the samples is evidence that *T. vaginalis* recovered from the host were in a high iron environment, responsible of the up-regulated expression of the AP65 surface adhesion (Garcia et al.,

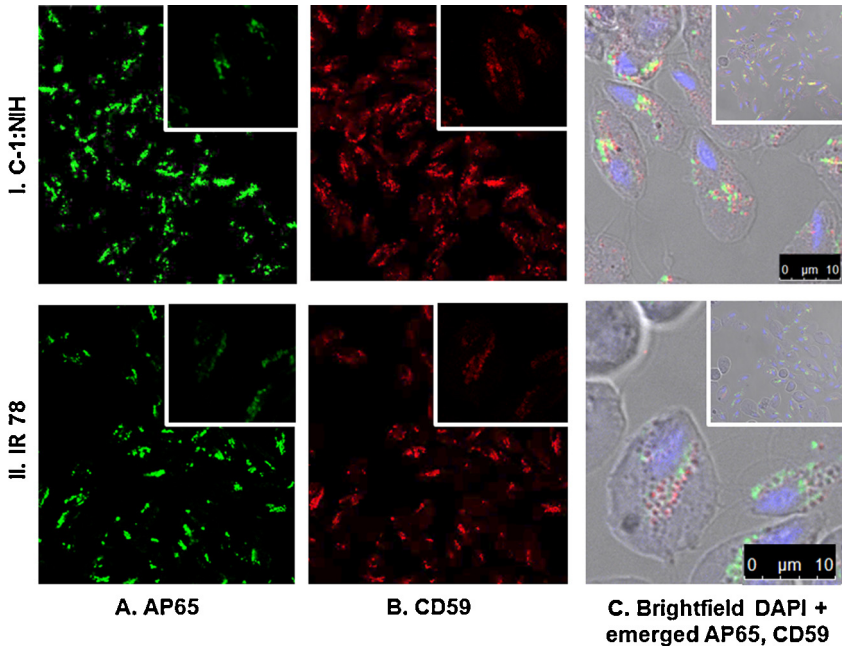

Fig. 4. Acquisition of CD59 by *T. vaginalis* parasites of isolates C-1:NIH (I) and IR 78 (II) grown in the presence of murine erythrocytes. Panel A shows surface AP65 with 12G4 MAb. Panel B evidences surface CD59 for organisms exposed to RBCs and probed with MAb to CD59. Panel C combines brightfield and confocal fluorescence of MAb of an enlarged view of the parasite exposed in the insets of panels A and B. The size scale is 10 µm.

2003). As above and throughout, the negative controls yielded no reactivity with trichomonads by fluorescence. These data suggest strongly that *T. vaginalis* parasites acquire and place on the surface mouse CD59 during intraperitoneal infection.

3.3. *Acquisition by* T. vaginalis *organisms of mouse CD59 from erythrocytes*

It has been shown that trichomonads hemagglutinate and lyse erythrocytes (Lehker et al., 1990) that then permits the parasites to grow and multiply in medium without serum (Lehker et al., 1990; Lehker and Alderete, 1992). Therefore, we wanted to confirm the presence of CD59 on RBCs to study whether the parasites can acquire CD59 from this source. Fig. 3 shows the brightfield of RBCs (panel A) used for fluorescence as positive control. The CD59 protein was readily visualized on RBCs as well as a larger macrophage seen in the preparation with MAb to CD59 (panel B).

This finding now permitted us to test for the acquisition of CD59 by parasites of isolates C-1:NIH and IR 78 grown in the presence of erythrocytes. Indeed, in a representative experiment, we show that trichomonads exposed to RBCs have strong fluorescence with MAb to CD59 (Fig. 4, panels B). Again, surface detection of AP65 with MAb 12G4 was evident (panels A). The pictures in panels C are the combined fluorescence in addition to brightfield of the parasites exposed to DAPI. Insets of enlarged parasites further demonstrate in detail the presence of AP65 and CD59 by confocal microscopy. This experiment reinforces the notion that *T. vaginalis* organisms can acquire CD59 from different cells, including RBCs, during infection.

3.4. *Surface-associated CD59 offers some level of protection against complement-mediated lysis*

T. vaginalis organisms are sensitive to complement-mediated lysis, and proteases are known to protect parasites from complement (Demes et al., 1988; Alderete et al., 1995). We now wanted to test whether the surface-bound mouse CD59 provides trichomonads with some level of protection against human complement. We felt this was possible because of the prior work that has confirmed the protective role of mouse CD59 against complement from human and rodents (Powell et al., 1997).

Parasites grown in batch culture in the presence or absence of erythrocytes were exposed to different amounts of human serum complement from 0.2 mg/ml to 1.2 mg/ml. After incubation for 1.5 h, the numbers of organisms were enumerated. Fig. 5 presents results for a representative experiment using *T. vaginalis* isolates C-1:NIH (part A) and IR 78 (part B). The number of parasites regardless of presence or absence of growth with RBCs but without complement after 1.5 h was normalized to 100% according to the specific activities (sp act) determined by the fluorimetric determinations. Data for each isolate using 8 replicates is presented and shows a complement concentration-dependent killing for both isolates. The statistical analysis indicates a C3 concentration-dependent correlation with surviving organisms grown in the presence of RBC ($p < 0.01$). For example, at 1.2 mg/ml complement for isolate C-1:NIH, there was 21.4% more live trichomonads from co-cultures with RBCs than control organisms (part A). Likewise IR 78 grown in the presence of RBC and incubated at 1.2 mg/ml complement had 23% more live organisms. Moreover, for this experiment and for both isolates, the coefficient of variation percentage (CV) for isolate C-1:NIH cultured with and without RBCs ranged from 9% to 20%, and for isolate IR 78 examined identically the CV was less than 17%. We next performed the Student's *t* test, and the results for the two isolates were found to be statistically significant ($p < 0.05$). These data illustrate that the CD59 acquired from host erythrocytes at least in part enhanced the ability of the parasites to resist lysis by complement.

4. Discussion

One of the main regulatory proteins to evade inappropriate membrane attack complex (MAC) lysis on autologous cells is protectin, which is also referred to as CD59. This molecule is an 18-kDa to 20-kDa GPI-linked membrane protein widely expressed on diverse cell surfaces (Meri et al., 1990), including endothelia and epithelia of the female reproductive tract (Oglesby et al., 1996). Male reproductive tract cells, such as prostate tissues, also have

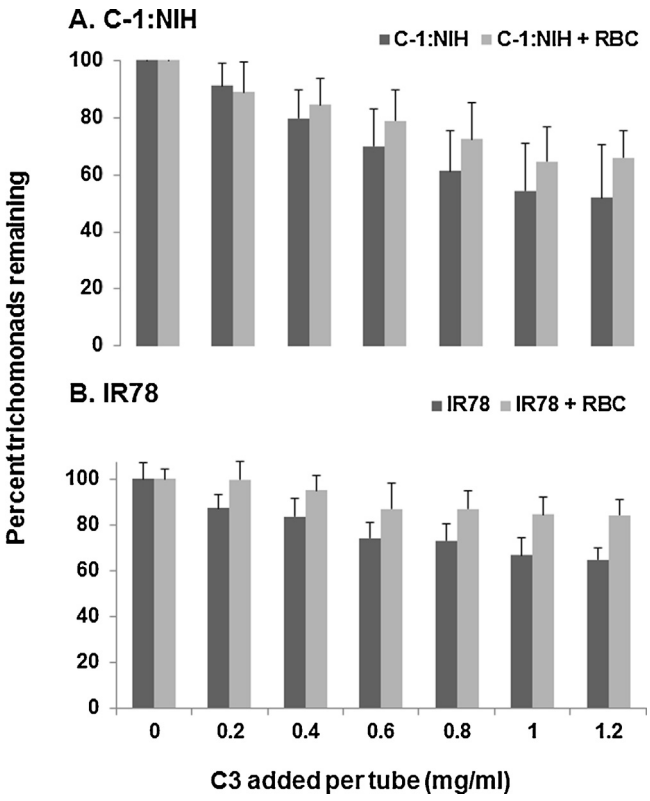

Fig. 5. Trichomonads of *T. vaginalis* isolates C-1:NIH (A) and IR 78 (B) derived from co-culture with (light bars) and without (dark bars) erythrocytes. The number of parasites regardless of presence or absence of growth with RBCs but without complement after 1.5 h was normalized to 100%. The specific activities (sp act) used for normalizing the results to 100% for the control C-1:NIH organisms without and with erythrocytes (A) were 23,682 and 7074, respectively. The sp act for the control IR 78 parasites without and with erythrocytes (B) was 13,434 and 5300, respectively. These sp act were the average of the eight replicates of the representative experiment used here. Significance values of 0.027 and 0.002 ($p < 0.05$) were obtained for both isolates using a one-tailed Student's *t*-test.

CD59. This protein prevents MAC formation by inhibiting C9 binding to C5b–C8 complex in the cell membrane and its subsequent polymerization for pore formation (Kimberley et al., 2007). As the complement system is one of the first and most important lines of host defense, many pathogens have evolved diverse mechanisms to avoid complement attack. Different strategies to evade MAC lysis are also based on removal of complement components by using regulatory proteins present on host cells, such as CD59.

Successful sexual transmission and host colonization by *T. vaginalis* is dependent to a great extent on the sequestration of host proteins (Peterson and Alderete, 1982, 1984a,b,c; Mundodi et al., 2008; Lama et al., 2009). It has been established that trichomonads bind numerous host proteins, such as plasminogen, fibronectin, laminin, collagen, and apolipoproteins, for example (Mundodi et al., 2008; Lama et al., 2009). Indeed, some of these acquired host proteins, e.g., plasminogen, have been defined as "host pathogenicity factors" (Sun et al., 2004). The ability to capture and place on the surface host CD59 appears to be an evolutionary hallmark for overcoming a major component of the overall immune system that deals with complement. We hypothesized that acquisition by *T. vaginalis* organisms of host CD59 would confer a level of protection of the parasite against lysis by complement. Our hypothesis does not suggest complete protection by CD59, and in fact we have shown that iron-regulated cysteine proteinases also inhibit lysis by C3 (Alderete et al., 1995). Our study shows for the first time that these parasites place on their surface CD59 acquired from the host, and trichomonads with mouse CD59 have higher levels of survival when exposed to human complement. Our results are consistent with a report that mouse CD59 is a functional analog of human CD59 being able to inhibit human complement lysis using mouse CD59-transfected human cells (Powell et al., 1997). These data indicate complementary protective mechanisms that ensure survival of the parasite during infection after exposure to complement (Fig. 5). Thus, consistent with the previous reports on acquisition of host proteins (Peterson and Alderete, 1982, 1984a,b,c; Mundodi et al., 2008; Lama et al., 2009), it is not surprising to have found appropriation of CD59 by *T. vaginalis* organisms. It is conceivable that trichomonads acquire additional factors restricting complement activity, such as DAF (Kooyman et al., 1995). It is equally likely that alternative factors and/or mechanisms explain the survival differences observed in the experiment with complement (Fig. 5). For example, as lipoproteins act as carriers of the GPI-CD59 (Vakeva et al., 1994), exposure to such CD59-containing lipoproteins could represent a source of both CD59 and lipids for *T. vaginalis* (Peterson and Alderete, 1982, 1984c). In this case the parasites would obtain growth factors and CD59 for protection from complement.

Although the exact mechanism by which trichomonads acquire CD59 from the host is unknown, the GPI lipid anchor is critical for CD59 sequestration from one cell to another (Kooyman et al., 1995). It is known, for example that GPI-linked CD59 and DAF, another complement regulatory protein, transfer readily from erythrocytes to endothelium (Kooyman et al., 1995). Thus, it is likely that the mechanism by which *T. vaginalis* acquires CD59 directly from cells during infection or in the interactions with erythrocytes also occurs through the GPI lipid moiety. It has been found that soluble CD59 without the GPI lipid moiety either does not associate with cells, or the GPI lipid anchor is needed for optimal functional activity (Vakeva et al., 1994; Meri et al., 1996). Thus, it is necessary to determine how CD59 is anchored to the parasites surface will be further investigated.

There are numerous reports of bacteria, viruses, and parasites overcoming complement by having CD59-like molecules via acquisition of complement inhibitors or by lysis of complement proteins directly (Albrecht et al., 1992; Würzner, 1999). For example, the Herpes virus, Hepatitis C virus, and HIV escape from complement via capsid incorporation of CD59 obtained from the host (Saifuddin et al., 1997; Amet et al., 2012; Ejaz et al., 2012). Further, although it has been suggested that parasites, such as *Naegleria fowleri*, *Schistosoma mansoni*, and *Entamoeba histolytica*, inhibit complement lysis by molecular mimicry with CD59-like surface proteins (Braga et al., 1992; Parizade et al., 1994; Fritzinger et al., 2006; De Taeye et al., 2013), to our knowledge experiments demonstrating inhibitory properties of these proteins have not been reported. Recent studies have proposed that *Helicobacter pylori* and *Escherichia coli* resistance to complement occurs by transference of CD59 from human cell surfaces to their bacterial membranes (Rautemaa et al., 2001).

The property of *T. vaginalis* acquisition of CD59 confers an advantage to the parasite, which, at the same time, also is to the detriment of the host concomitant with protection of the organisms to host complement. CD59 sequestration by the trichomonads may result in the depletion of this protective protein from the surface of host cells and tissues. This will make the host cells and tissues vulnerable to exposure to complement-mediated damage. This lysis of host cells and RBCs would provide nutrients such as for lipids and iron to the parasite (Peterson and Alderete, 1984b,c; Lehker et al., 1990; Lehker and Alderete, 1992). Given the inflammatory nature of trichomonosis and severe irritation coupled with strawberry cervix, it is possible that erythrocytes can be found within the vagina during severe infections caused by *T. vaginalis* (Alderete et al., 1992) presenting the parasite with a reservoir of CD59 for protection during menstruation when complement is present in the vagina.

In summary, this property of acquisition of CD59 from the host by trichomonads appears to be a novel and additional,

complementary mechanism of immune evasion. This co-evolutionary adaptation between *T. vaginalis* and the host permits that such accommodation between them would take advantage of the dual role of the efficient immune surveillance and homeostasis of complement that is exploited by both host and parasite (Ricklin et al., 2010). The organism would harness and take advantage of the host's own protective systems in a holistic fashion for its own life-long persistence. Once again *T. vaginalis* appears to have availed itself of a novel, alternative host-like mechanism of immune evasion.

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