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Development and characterization of mouse monoclonal antibodies to eight human complement components: Analysis of reactivity with orthologs of nine mammalian genera

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ABSTRACT

To study complement function in mammalian leishmanioses, we developed mouse monoclonal antibodies to the human complement system components C1q, C4, factor D, factor H, factor B, properdin, C5 and C9. Antibody specificity was determined by indirect and capture ELISA and by Western blot. In flow cytometry analysis, seven antibodies recognized the cognate component on human serum-opsonized *Leishmania* promastigotes. Antibody reactivity was screened against promastigotes opsonized with sera of nine mammalian genera: pig, guinea pig, goat, rabbit, cat, dog, hamster, jird and rat. No antibody recognized jird epitopes on promastigotes. Anti-C4, -properdin, and -C5b reacted with the orthologous protein of all other mammals tested except cat (anti-properdin) and hamster (anti-C5b); anti-C9 only recognized the rabbit ortholog, and anti-C1q, -factor B and -factor H did not react with any of the nine orthologs. Such interspecies crossreactive antibodies can be valuable tools for analysis of mammalian complement function in infectious diseases.

1. Introduction

Approximately 70% of emerging pathogens that affect humans are zoonotic [1]. Most leishmanioses are also zoonoses caused by trypanosomatid parasites of the genus *Leishmania* transmitted to mammalian hosts by a sandfly vector during the blood meal [2]. In contact with host blood or tissue humors, promastigotes activate complement, which triggers parasite endocytosis and promotes infection, or the lytic cascade that kills the parasite. In *Leishmania* infection, host complement thus has a decisive role in early parasite survival or death [3].

Complement is a principal component of the innate immune system, which is involved in immune surveillance, host homeostasis, and induction of adaptive immune responses [4]. The complex complement system comprises fluid-phase and cell-bound proteins that include cellular and plasmatic pattern recognition molecules, serum zymogens, cell-bound receptors, and regulatory elements; in all, approximately 50 components [5]. In mammals, complement has various physiological activities; it mediates host defense by direct lysis of foreign cells, it opsonizes microorganisms for phagocytosis, and it induces and regulates adaptive immune responses [6]. Complement also participates in tissue pruning and regeneration [7], and in apoptotic cell and immune complex disposal, thus preventing tissue injury and development of immunopathologies [8].

Complement can be activated by three independent routes, i) by antibodies, ii) by lectins (ficolins and mannan-binding lectins; MBL), or iii) by direct binding of the activated complement C3 form, C3 (H_2O), to permissive microbial and cellular surfaces. These routes are known as the classical (CP), lectin (LP), and alternative (AP) complement activation pathways, respectively. Activation of CP and LP lead to assembly of the C4b2a complex (CP C3 convertase), which activates C3 and deposits C3b on foreign surfaces and tags them covalently for subsequent phagocytosis or lysis [9].

Abbreviations: CP, classical complement pathway; LP, lectin complement pathway; AP, alternative complement pathway; c-ELISA, capture ELISA; fB, factor B; fD, factor D; fH, factor H; fI, factor I; i-ELISA, indirect ELISA; mAb, monoclonal antibody; MBL, mannan binding lectin

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The AP is activated by the spontaneous C3 hydrolysis in plasma, giving rise to C3(H_2O), which reacts with hydroxyl and amino groups of microbial or apoptotic cell surfaces. Complement factor B (fB) binding to C3(H_2O) forms the C3(H_2O)B proconvertase; this is cleaved by factor D (fD) to become C3bBb, the AP C3 convertase that catalyzes a very active C3b amplification loop. Properdin binding to AP C3 convertase forms a C3bBbP, a multicomponent complex with a longer half-life that amplifies C3 activation [10]. To limit excessive C3 deposition, which can generate tissue-damaging products, assembly of AP C3 convertases on mammalian cells is strictly controlled by regulatory components such as membrane cofactor protein (MCP/CD46), complement receptor type-1 (CR1) and decay accelerating factor (DAF/CD55), all of which promote enzyme decay. In plasma, C3 activation is controlled by factor H (fH) and factor I (fI). fH competes with fB for C3b binding and disrupts assembly of the AP C3/C5 convertases. In addition, fH acts as cofactor for fI in cleavage of C3b to inactive iC3b [11]. Binding of additional C3b molecules to C4b2a and C3bBb generates the CP and AP C5 convertases. These enzymes cleave C5 to generate C5b, which initiates assembly of the lytic complement cascade; subsequent C6 and C7 binding forms a hydrophobic C5b67 complex that inserts into the target membrane. Addition of C8 and C9 complete the lytic cascade by forming an amphipathic complex that kills C3b-tagged cells [12].

To monitor the time course of complement activation pathways in *Leishmania* infection, we generated murine mAb to five human complement components (C1q, C4, C5, C9, and properdin) and three factors (H, B, and D), and used a *Leishmania* opsonization assay to select for antibodies to determinants of physiologically activated complement components. In zoonotic infections, most reservoir hosts are non-human mammals [13]. To facilitate functional complement studies with other mammalian species and zoonotic diseases, we defined the cross-reactivity of these anti-human complement mAb to orthologous complement components of nine mammalian genera.

2. Materials and methods

2.1. Parasites

Promastigotes of *Leishmania amazonensis* María (MHOM/SD/43/124) and *L. infantum* (M/CAN/ES/97/10,445) zymodeme MON-1 were cultured at 27 °C in RPMI 1640 medium with 10% fetal bovine serum (FBS) [14]. Stationary phase parasites were harvested by centrifugation (1500 x g, 15 min, 20 °C), washed twice in PBS pH 7.2, and the concentration adjusted.

2.2. Animal sera

Blood was obtained from *Meriones unguiculatus* (jird), *Cavia porcellus* (guinea pig), *Rattus norvegicus* (rat), *Mesocricetus auratus* (Syrian hamster), *Canis familiaris* (dog), *Felis domestica* (cat), *Capra aegagrus* (goat), *Sus scrofa* (pig) and *Oryctolagus cuniculus* (rabbit). Human serum was from healthy donors who gave written consent. Blood was allowed to clot in siliconized glass tubes (30 min, 20 °C), and serum aliquots were stored in liquid nitrogen.

2.3. Antibodies, cell culture media and reagents

Experiments were approved by the Ethics Committee of the Instituto de Salud Carlos III (CEI PI 12-2009). Purified human complement proteins C1q, C4, C5, C9, properdin, fB, fH and fD were purchased from Calbiochem (Nottingham, UK), bovine serum albumin (BSA), Freund's incomplete (IFA) and complete (CFA) adjuvants, aminopterin, polyethylene glycol 1500 (PEG 1500), o-phenylene diamine (OPD), 3, 3-diaminobenzidine (DAB) and Tween-20 were from Sigma-Aldrich (St. Louis, MO) and H_2O_2 from PanReac AppliChem (Barcelona, Spain). FBS was from Hyclone (GE Healthcare, Marlborough, MA), Clonacell-HY medium-E from Stem Cell (Grenoble, France), RPMI 1640 from Lonza (Verviers, Belgium), sulfo-NHS-LC-biotin from Pierce (Thermo Fisher Scientific), Maxisorp 96-well microtiter plates from Nunc (442404; Roskilde, Denmark). Goat anti-mouse IgG (H + L), IgG_1, IgG_{2a}, IgG_{2b} and IgG_3, *kappa* and *lambda* chains, all conjugated to horseradish peroxidase (HRP), and streptavidin HRP were from Southern Biotechnology (Birmingham, AL) and fluorescein isothiocyanate (FITC)-goat anti-mouse IgG was from Jackson ImmunoResearch (West Grove, PA).

2.4. Mouse immunization and cell hybridization

Eight- to twelve-week-old female BALB/c mice ($n = 3$) were immunized with three intraperitoneal (i.p.) injections, two weeks apart, with human C1q, C4, fB, fH, fD, properdin, or C9 proteins, and DBA/2 mice ($n = 3$) with C5 protein. The first injection (prime) consisted of 50 μg immunogen/0.1 ml emulsified in CFA, and the two boosts were 25 μg immunogen/0.1 ml emulsified in IFA. One week after the last injection, mice were bled and antibody titer determined. Four days before cell hybridization, donor mice received a final intravenous (i.v.) boost with 25 μg immunogen/0.1 ml PBS. Mouse myeloma Sp2/0-Ag-14 cells were cultured in RPMI 1640 with 10% FBS. Donor mouse spleen cells were fused with Sp2/0-Ag-14 cells at a 3:1 ratio, using PEG 50% (w/v) and standard procedures [15]. Clonacell-HY aminopterin medium was used for hybridoma selection. Cell culture supernatants were screened for antibody reactivity and cells from positive wells cloned twice by limiting dilution in Clonacell HY medium.

2.5. Immunoassays

Serum antibody was titrated and hybridoma supernatants were screened by i-ELISA, c-ELISA, Western blot and flow cytometry.

i-ELISA was used to screen for antibody activity in hybridoma supernatants. Plates were coated with 50 μl of analyte (C1q, C4, fD, fB, fH, properdin, C5 or C9) (1 μg/ml PBS; 2 h, 37 °C or 16 h, 4 °C). Plates were washed three times with PBS- 0.05% Tween 20 (PBST) and blocked (30 min, 37 °C) with 75 μl 2% BSA, washed three times with PBST, and 50 μl/well hybridoma supernatant added (1 h, 37 °C). Mouse pre-immune serum at 1/500 dilution was used as negative control. Plates were washed three times with PBST and 50 μl HRP-goat anti-mouse IgG (1:2000) was added and incubated (30 min, 20 °C). Plates were washed five times with PBST, followed by 100 μl/well OPD/H_2O_2 (1 mg/ml OPD in 0.2 M disodium phosphate-0.1 M citric acid pH 4.9, with 1/1000 v/v H_2O_2) and incubation (5 min, 20 °C, in the dark). The reaction was terminated using 50 μl/well 3 N H_2SO_4 and absorbance was recorded at $OD_{492\ nm}$ on an Anthos 2020 microtiter plate reader.

For c-ELISA, plates were coated with 50 μl goat anti-mouse immunoglobulin G (H + L) (3 μg/ml PBS; 16 h, 4 °C), blocked with 75 μl 2% BSA (30 min, 37 °C), and washed 3 times with PBST. Culture supernatants (50 μl) were added and incubated (2 h, 37 °C), wells were washed with PBST, followed by 50 μl biotin-labeled normal human serum (biotin-NHS) [16] (1/100 in PBS-2% BSA-0.05% Tween 20; 1 h, 20 °C); mAb SIM 180-13.4.1 which does not react with human complement components was used as negative control. Plates were washed with PBST and 50 μl streptavidin-HRP (1:2000 in PBST) were added (30 min, 20 °C), then washed five times with PBST and the enzyme reaction developed as above.

mAb isotypes were determined by c-ELISA. Plate Wells were coated with 50 ul/well goat anti-mouse IgG (3 μg/ml in PBS; 2 h, 37 °C or 16 h, 4 °C), and washed thoroughly in PBST. Hybridoma supernatants (50 μl/well) were added and incubated (1 h, 37 °C). Plates were washed three times with PBST and 50 μl HRP-conjugated goat anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, *kappa* or *lambda* chains were added (1:2000 in PBST) and incubated (30 min, 20 °C). Plates were washed three times with PBST and the peroxidase reaction developed.

For Western blot analysis, purified commercial complement proteins (1 μg) were resolved in non-reducing and reducing conditions in

SDS-PAGE (7.5% for fH, C4, C5; 10% for C1q, C9, properdin, fB; 12.5% for fD). Proteins were transferred to nitrocellulose membranes, which were incubated (20 min, 20 °C) in PBS with 5% skim milk, and washed three times inPBST. Hybridoma supernatants were added and incubated (16 h, 4 °C, with rocking). Membranes were washed three times with PBST and incubated with HRP-goat anti-mouse IgG (1 h, 20 °C). After two wash with PBST and one with PBS, antibody-reactive bands were visualized by incubation in DAB (1 mg/ml)/H_2O_2 (1:2000) reagent.

For flow cytometry analyses, *L. amazonensis* and *L. infantum* promastigotes were used with identical results; *L. amazonensis* results are shown. A suspension of *L. amazonensis* promastigotes (50 µl, 2 × 10 [8] cells/ml) was mixed with 50 µl of 50% PBS-diluted mammalian serum and incubated (10 min, 37 °C); as negative control we used the same serum diluted in PBS/10 mM EDTA. The reaction was terminated using 1 ml PBS with 1% paraformaldehyde. Promastigotes were washed twice in PBS by sedimentation (11,000 x g, 1 min, room temperature). Opsonized parasites were incubated (1 h, 20 °C) with monoclonal anti-C1q (SIM 19413.2.1), -C4 (SIM 192-4.2.1), -fB (SIM 384-42.12.1), -properdin (SIM 246-1.1.1), -fD (SIM 234-5.12.1), -C5b (SIM 225-3.2.1), -C9 (SIM 235-35.16.7) or -fH (SIM 214-12.3.8) antibodies, or with isotype-matched mAb control anti-*Clostridium perfringens* toxin: SIM 438-11.3.2 (IgG1), SIM 438-60.3.2 (IgG2a) and SIM 438-9.3.2 (IgG2b). Promastigotes were washed with PBS and incubated with FITC-goat anti-mouse IgG (30 min, 20 °C, in the dark). Parasite-bound fluorescence was measured in a flow cytometer (FACScalibur; Becton Dickinson, San José, CA). Parasites were gated by forward scatter (FSC) vs. side scatter (SSC) and plotted independently in a secondary plot of SSC vs. FL1 (green, 530 nm) after excitation with a 488 nm argon ion laser.

To analyze protein similarity of complement, orthologous sequences were obtained from the NCBI protein database, and the percentage of identity calculated by amino acid alignment using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins).

3. Results

3.1. Development and selection of mAb to human complement components

From 30 hybridizations, we generated 231 mAb to eight human complement proteins (C1q, C4, fD, fB, fH, properdin, C5 and C9). Specificity was determined by i-ELISA, c-ELISA and Western blot against purified complement components, and by flow cytometry with human serum-opsonized *Leishmania* promastigotes. We selected one representative mAb clone for each complement protein, and tested crossreactivity with orthologous proteins of nine mammalian genera by flow cytometry with serum-opsonized promastigotes. Immunogens, cell fusions and hybrids, isotype and reactivity in c- and i-ELISA are summarized in Table 1. As control, we used the anti-human C3 α-chain mAb SIM 27-49 (IgG2b) [17].

3.2. Western blot detection of mAb reactivity to purified complement components

Purified human complement proteins were separated by SDS-PAGE in non-reducing and reducing conditions (Fig. 1), transferred to nitrocellulose membranes, and probed with culture supernatant of the representative hybridoma clones. In non-reducing conditions, all mAb recognized the cognate complement component; the anti-C1q and -C4 mAb reacted with additional bands caused by aggregation/fragmentation of the purified protein. In reducing conditions, anti-C1q, -fB, and -fD mAb showed the predicted reactivity. The anti-C1q mAb recognized a Mr ~21,000 protein band that can be attributed to the C1q C-chain. The anti-fB mAb reacted with a Mr ~64,000 protein; as fB is digested to Ba (Mr ~30,000) and Bb (Mr ~60,000) polypeptides, the anti-fB mAb probably reacted with a Bb epitope. fD is a single chain molecule of Mr ~24,400; in non-reducing conditions, the anti-fD mAb reacted with a Mr ~22,000 band that increased to Mr ~26,000 in reducing conditions, similar to the reduction induction mobility decrease for fH [18]. In reducing conditions, the antiproperdin, -C9, -C5, -fH, and -C4 mAb did not recognize the cognate antigens in Western blot.

3.3. Flow cytometry analysis of mAb reactivity to promastigote-bound complement component

To assess mAb specificity, we measured their reactivity to epitopes exposed by complement components deposited on normal human serum (NHS)-opsonized *L. amazonensis* promastigotes. Parasites were opsonized by incubation with 50% NHS; as negative control, promastigotes were opsonized in 10 mM EDTA-treated NHS, which blocks complement activation [17]. After opsonization, promastigotes were washed, fixed and aliquots incubated with each of the eight mAb; SIM 27-49 anti-C3α mAb was included as positive control. Following incubation, promastigotes were washed and incubated with FITC-goat anti-mouse IgG. Finally, promastigotes were washed and parasite-bound fluorescence measured by flow cytometry.

Plotting promastigote numbers vs. mAb fluorescence intensity indicated that seven of the eight mAb clearly bound to the immunizing complement component (Fig. 2). For anti-C4, -fH, -C5b, -C9, -C3b, and -C1q, profiles showed a bell-shaped, near-symmetric distribution that spans one log fluorescence intensity, which suggested homogeneous distribution of the bound epitopes; these profiles contrasted with the broad, asymmetric distribution over two logs of fluorescence intensity obtained with anti-fB and anti-properdin mAb which suggested a heterogeneous deposit of Bb and properdin epitopes. Because C1q binding to promastigotes is not Ca^{2+}-dependent, mAb binding to NHS- and NHS/EDTA-opsonized promastigotes was similar. fD is a fluid-phase component that does not bind to promastigotes; anti-fD binding thus cannot be measured and are not shown.

Table 1
Hybridization assays, name and characteristics of mouse mAb to human complement components.

Immunogen	Fusion number	Hybrids obtained	Representative mAb	Isotype	c-ELISA (A_{492} nm)	i-ELISA (A_{492} nm)
C1q	2	15	SIM 194-13.2.1	IgG2a/κ	2.27/0.6[a]	> 3.5/0.08[a]
C4	4	50	SIM 192-4.2.1	IgG2b/κ	2.47/0.5	3.03/0.3
Factor B	6	43	SIM 384-42.12.1	IgG1/κ	> 3.5/0.3	> 3.5/0.1
Properdin	3	27	SIM 246-1.1.1	IgG1/κ	> 3.5/0.6	2.21/0.4
Factor D	3	19	SIM 234-5.12.1	IgG1/κ	3.37/0.5	2.65/0.1
C5	8	43	SIM 225-3.2.1	IgG1/κ	> 3.5/0.5	3.36/0.08
C9	2	26	SIM 235-35.16.7	IgG1/κ	3.23/0.6	2.62/0.1
Factor H	2	8	SIM 214-12.3.8	IgG2b/κ	> 3.5/0.5	> 3.5/0.1

c-ELISA: Capture ELISA; i-ELISA: Indirect ELISA; mAb, monoclonal antibodies.
[a] O.D. $_{492}$ nm: positive signal/background signal.

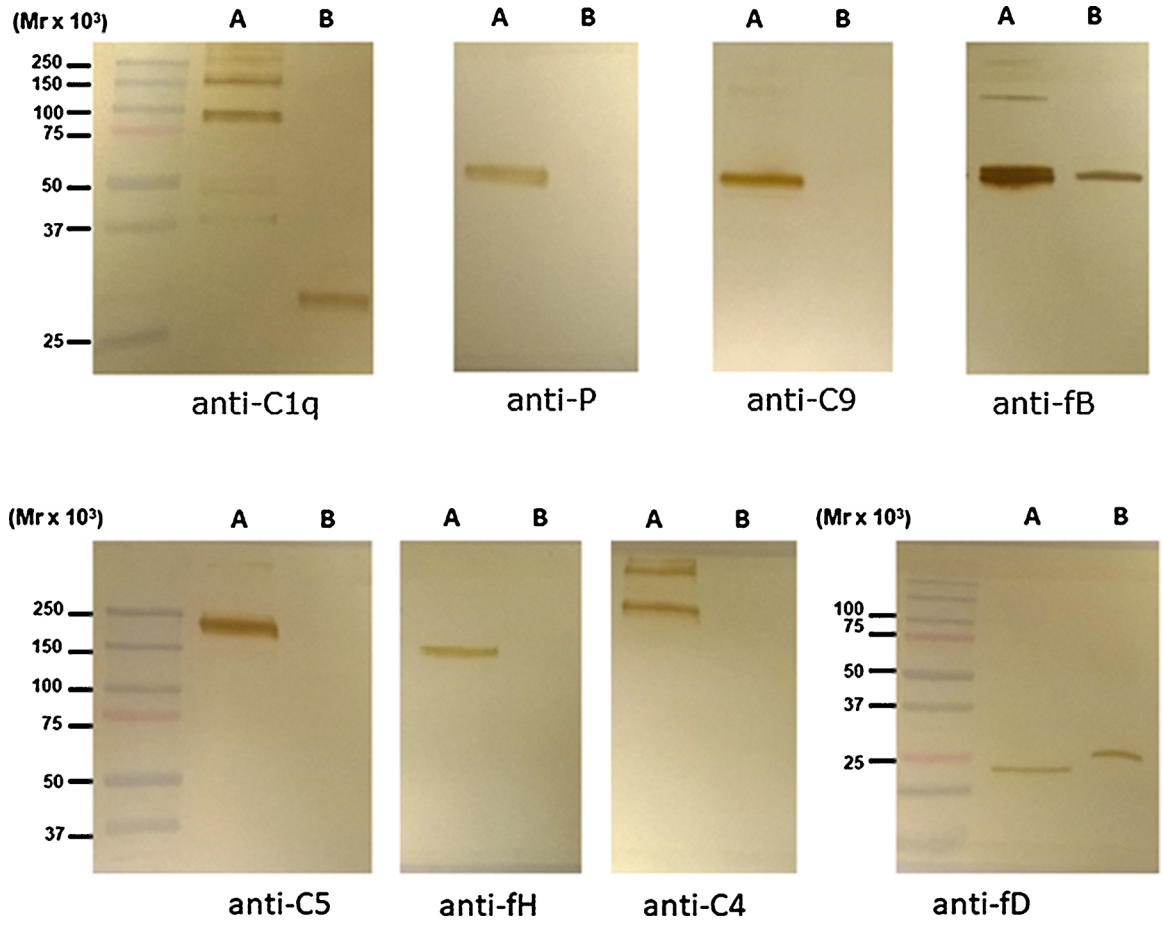

Fig. 1. Analysis of mAb reactivity by Western blot against purified complement components. Proteins were electrophoresed under non-reducing (lane A) or reducing (lane B) conditions, transferred to nitrocellulose membranes and probed with mAb culture supernatants.
Bound mAb were detected with HRP-goat anti-mouse IgG. For C1q, properdin, C9 and fB, 10% acrylamide was used; for C5, fH, and C4, 7.5%; for fD, 12.5%.

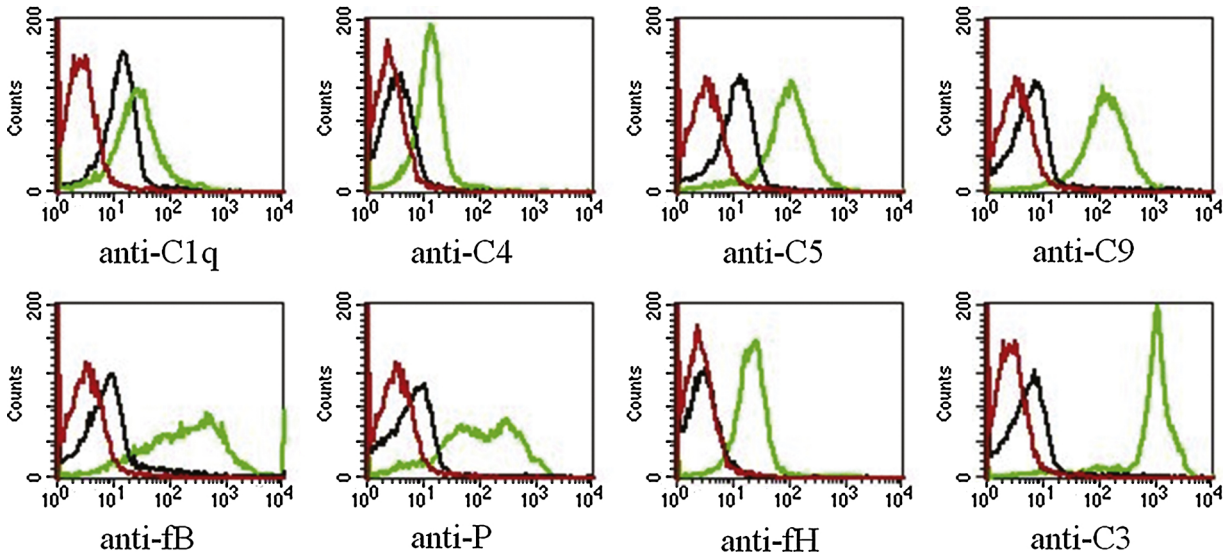

Fig. 2. Flow cytometry analysis of mAb reactivity to normal human serum (NHS)-opsonized promastigotes. Promastigotes were opsonized (10 min, 20 °C) with 50% NHS; control parasites were opsonized in 10 mM EDTA-treated 50% NHS. After incubation, promastigotes were washed, fixed in PBS-containing 1% paraformaldehyde, and incubated with mAb to complement components, or with isotype-matched mAb control. After incubation, bound complement was detected with FITC-goat anti-mouse IgG. Y-axis: number of promastigotes; X-axis: fluorescence intensity signal. Promastigotes opsonized with 50% NHS and incubated with mAb to complement components (green), isotype-matched mAb control (red); promastigotes opsonized with 50% NHS/EDTA and incubated with mAb to complement components (black).

Table 2
Flow cytometry analysis of mAb reactivity to promastigote-bound orthologues of nine mammalian genera.

	Anti-C1q	Anti-C4	Anti-fB	Anti-P	Anti C5b	Anti C9	Anti- fH	Anti-C3
Rabbit	– (78)[a]	44/7 (84)[b]	– (86)	102/7 (79)	17/7 (84)	129/7 (72)	– (66)	– (42)
Goat	– (76)	13/4 (80)	– (81)	240/4 (75)	263/4 (80)	– (69)	– (62)	– (43)
Pig	– (76)	29/3 (80)	– (80)	70/4 (77)	21/4 (81)	– (72)	– (63)	– (73)
Guinea Pig	– (74)	93/3 (78)	– (81)	210/7 (74)	122/17 (82)	– (68)	– (60)	– (74)
Dog	– (82)	52/5 (82)	– (81)	110/6 (75)	139/6 (75)	– (70)	– (64)	– (43)
Rat	– (76)	8/2 (76)	– (85)	33/5 (79)	31/5 (80)	– (66)	– (64)	– (76)
Cat	– (74)	169/6 (85)	– (82)	– (76)	81/6 (83)	– (73)	– (64)	– (71)
Hamster	– (78)	14/5 (79)	– (85)	125/6 (81)	– (80)	– (66)	– (61)	– (76)
Jird	– (71)	– (79)	– (85)	– (77)	– (79)	– (64)	– (43)	– (76)

[a] () Percent protein identity between human and animal ortholog proteins (http://www.ncbi.nlm.nih.gov/BLAST/).
[b] Mean fluorescence intensity of specific mAb / Mean fluorescence intensity of isotype-matched mAb control.

3.4. mAb crossreactivity with complement of nine mammalian genera

Promastigotes were opsonized with pig, goat, guinea pig, rabbit, cat, hamster, jird, dog, and rat sera and analyzed by flow cytometry with the seven mAb positive in the previous assay. Promastigotes opsonized with human serum were used as positive control. None of the seven mAb recognized jird complement epitopes on promastigotes (Table 2). Three mAb (anti-C4, -properdin and -C5) bound the epitope of the orthologous component of all genera, with the exceptions of anti-properdin with cat serum, and anti-C5 with hamster serum. In most cases, anti-fB, -C9 and -fH mAb did not react with the epitope of the orthologous protein, despite the considerable amino acid sequence identity ($\geq$60-80%) of human and non-human mammalian complement. The anti-C9 mAb recognized only rabbit epitopes, and anti-C1q, -fB and -fH did not react with any of the nine proteins tested. As the *L. amazonensis* promastigote surface activates complement efficiently [19], this lack of reactivity can be attributed to mAb failure to recognize the non-human C1q, fH, fB and C9 determinants on promastigotes.

4. Discussion

In human and other mammalian models, *Leishmania* invasion is counteracted by natural antibodies and complement, the first anti-parasite innate immune mechanisms activated, within seconds of promastigote contact with host blood [20]. mAb to complement components are thus tools with which to study complement function and define host factors that contribute to susceptibility to the parasite. Such analyses can be of particular use in mammalian clades with high zoonotic potential as reservoirs, such as lagomorphs (rabbits), rodents (guinea pig, rat, hamster, jird), carnivores (dog, cat) and artiodactyls (goat, pig) [12,21]. Some members of these groups share living habitat with humans, and depending on environmental conditions [1,22], can act as wild (jird), peridomestic (rabbit) or domestic (dog, cat) reservoirs of *Leishmania* spp [23–27].

To study complement activation in non-human mammals, we developed mAb against eight human complement proteins. mAb reactivity was selected on the principle that antibodies that bind their cognate antigen in c-ELISA react mainly with native antigenic determinants [28,29], and mAb selected in a *Leishmania* opsonization assay would recognize epitopes generated after physiological activation of complement. The anti-C4, -properdin and -C5b mAb bound ~98% of orthologous protein epitopes on opsonized promastigotes, except cat (anti-properdin), and hamster (anti-C5b). Five mAb (anti-properdin, -C9, -C5, -C4, -fH) recognized conformational epitopes that are destroyed by reduction in SDS-PAGE and Western blot (Fig. 1). None of the mAb to human complement components reacted with jird complement; jirds and mice (the mAb host species) belong to the family Muridae, and their sera behave similarly in *Leishmania* opsonization (not shown). Their close phylogenetic relationship might thus explain the failure of the murine antibodies to react with jird complement.

In contrast, the anti-C1q, -fB, -fH and -C9 mAb did not react with the complement orthologues of the nine mammals studied (except anti-C9 with rabbit C9). Given the substantial identity of C1q (71–82%), fB (80–86%) and fH (43–66%) proteins among these genera (Table 2), a certain degree of mAb crossreactivity would be predicted with C1q, fB and fH epitopes of non-human mammals. In addition, as fB and fH have crucial regulatory roles in AP activation, one could anticipate that selection would limit molecular divergence of structure and function. C3 proteolysis triggers conformational changes in C3b [30–33]; fB and fH interaction with C3b might uncover private neoepitopes in both factors, which could explain the lack of mAb recognition of orthologous epitopes. Nonetheless, as all the anti-complement mAb were obtained by immunization with the native protein, their induction was triggered by epitopes expressed prior to fB and fH assembly into C3b, or C3bBb/C3bBbC3b complexes.

The anti-human C3α, mAb SIM 27-49, raised against human C3 and used here as a control, is a similar case. Protein identity of C3α polypeptides of human and non-human mammals ranges from 42% to 76%. Despite this substantial similarity SIM 27-49 did not react with any of the C3b orthologues tested on opsonized promastigotes. SIM 27-49 reacted directly with surface-bound C3b determinants, and neoepitopes cannot be formed by secondary protein interactions. mAb raised to native antigenic determinants of human C1q, fB, fH, C9 and C3 are specific for the eliciting epitope alone, and the lack of crossreactivity with other mammalian orthologues could be due to their antigenic variability. Note that the lack of antibody crossreactivity was observed only for those components with a central role in activation (C3) and regulation (fH, fB) of complement pathways.

In conclusion, we consider that crossreactive mAb to mammalian complement components are of use for studying infection mechanisms in mammalian hosts, and can provide valuable information about the infection strategies of zoonotic agents and the innate immune response of their reservoir hosts. They could contribute to the development of immune assays to monitor mammalian complement reactions in normal and pathological conditions.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cimid.2018.11.008.

References

[1] L.H. Taylor, S.M. Latham, M.E.J. Woolhouse, Risk factors for human disease emergence, Phil. Trans. R. Soc. Lond. B 356 (2001) 983–989.

[2] A.L. Bañuls, M. Hide, F. Prugnolle, Leishmania and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans, Adv. Parasitol. 64 (2007) 1–109.

[3] I. Moreno, M. Domínguez, D. Cabañes, C. Aizpurua, A. Toraño, Kinetic analysis of ex vivo human blood infection by Leishmania, PLoS Negl. Trop. Dis. 4 (2010) e743, https://doi.org/10.1371/journal.pntd.0000743.

[4] C.H. Nielsen, E.M. Fisher, R.G.Q. Leslie, The role of complement in the acquired immune response, Immunology 100 (2000) 4–12.

[5] D. Ricklin, G. Hajishengallis, K. Yan, J.D. Lambris, Complement: a key system for immune surveillance and homeostasis, Nat. Immunol. 11 (2010) 785–797.

[6] J.R. Dunkelberger, W. Song, Complement and its role in innate and adaptive immune responses, Cell Res. 20 (2010) 34–50.

[7] A.H. Stephan, B.A. Barres, B. Stevens, The complement system: an unexpected role in synaptic pruning during development and disease, Annu. Rev. Neurosci. 35 (2012) 369–389.

[8] D.S. Cole, P. Morgan, Beyond lysis: how complement influences cell fate, Clin. Sci. 104 (2003) 455–466.

[9] C. Speth, W.M. Prodinger, R. Würzner, H. Stoiber, M.P. Dierich, Complement, in: W.E. Paul (Ed.), Fundamental Immunology, sixth edition, Wolters Kluwer, 2008, pp. 1047–1078.

[10] L. Kouser, M. Abdul-Aziz, A. Nayak, C.M. Stover, R.B. Sim, U. Kishore, Properdin and factor H: opposing players on the alternative complement pathway "see-saw", Front. Immunol. 4 (2013) 1–11, https://doi.org/10.3389/fimmu.2013.00093.

[11] V.M. Holers, Complement and its receptors: new insights into human disease, Annu. Rev. Immunol. 32 (2014) 433–459.

[12] C. Bayly-Jones, D. Bubeck, M.A. Dunstone, The mystery behind membrane insertion: a review of the complement membrane attack complex, Phil. Trans. R. Soc. B 372 (2017) 201602.

[13] B.A. Han, A.M. Kramer, J.M. Drake, Global patterns of zoonotic diseases in mammals, Trends Parasitol. 32 (2016) 565–577.

[14] I. Moreno, J. Álvarez, N. García, S. de la Fuente, I. Martínez, E. Marino, A. Toraño, J. Goyache, F. Vilas, L. Domínguez, M. Domínguez, Detection of anti-*Leishmania infantum* antibodies in sylvatic lagomorphs from an epidemic area of Madrid using the indirect immunofluorescence antibody test, Vet. Parasitol. 199 (2014) 264–267.

[15] J.W. Goding, Antibody production by hybridomas, J. Immunol. Methods 39 (1980) 285–308.

[16] R. Schuh, E. Kremmer, E. Ego, M. Wasiliu, S. Thierfelder, Determination of monoclonal antibody specificity by immunoadsorption and western blotting, J. Immunol. Methods 152 (1992) 59–67.

[17] M. Domínguez, A. Toraño, Immune adherence–mediated opsonophagocytosis: the mechanism of Leishmania Infection, J. Exp. Med. 189 (1999) 25–35.

[18] R.B. Sim, R.G. DiScipio, Purification and structural studies on the complement-system control protein β_1H (factor H), Biochem. J. 205 (1982) 285–293.

[19] M. Dominguez, I. Moreno, M. López-Trascasa, A. Toraño, Complement interaction with trypanosomatid promastigotes in normal human serum, J. Exp. Med. 195 (2002) 451459.

[20] M. Domínguez, I. Moreno, C. Aizpurua, A. Toraño, Early mechanisms of Leishmania infection in human blood, Microbes Infect. 5 (2003) 507–513.

[21] B.A. Han, A.M. Kramer, J.M. Drake, Global patterns of zoonotic diseases in mammals, Trends Parasitol. 32 (2016) 565–577.

[22] J. Bradbury, Beyond the fire-hazard mentality of medicine: the ecology of infectious diseases, PLoS Biol. 1 (2003) 148–151.

[23] R.W. Ashford, The leishmaniases as emerging and reemerging zoonoses, Int. J. Parasitol. 30 (2000) 1269–1281.

[24] M. Gramiccia, L. Gradoni, The current status of zoonotic leishmaniases and approaches to disease control, Int. J. Parasitol. 35 (2005) 1169–1180.

[25] C. Maia, L. Campino, Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis? Trends Parasitol. 27 (2011) 341–344.

[26] J. Millán, S. Zanet, M. Gomis, A. Trisciuoglio, N. Negre, E. Ferroglio, An investigation into alternative reservoirs of canine leishmaniasis on the endemic island of Mallorca (Spain), Transboundary Emerg. Dis. 58 (2011) 352–357.

[27] R. Sobrino, E. Ferroglio, A. Oleaga, A. Romano, J. Millán, M. Revilla, M.C. Arnal, A. Trisciuoglio, C. Gortázar, Characterization of widespread canine leishmaniasis among wild carnivores from Spain, Vet. Parasitol. 155 (2008) 198–203.

[28] A.D. Smith, J.E. Wilson, A modified ELISA that selectively detects monoclonal antibodies recognizing native antigens, J. Immunol. Methods 94 (1986) 31–35.

[29] C. Schwab, H.R. Bosshard, Caveats for the use of surface-adsorbed protein antigen to test the specificity of antibodies, J. Immunol. Methods 147 (1992) 125–134.

[30] B.J.C. Janssen, E.G. Huizinga, H.C. Raaijmakers, A. Roos, M.R. Daha, K. NilssonEkdahl, B. Nilsson, P. Gros, Structures of complement component C3 provide insights into the function and evolution of immunity, Nature 437 (2005) 505–511.

[31] N. Nishida, T. Waltz, T.A. Springer, Structural transitions of complement component C3 and its activation products, Proc. Nat. Acad. Sci. U. S. A. 103 (2006) 19737–19742.

[32] J. Wu, Y.-Q. Wu, D. Ricklin, B.J.C. Janssen, J.D. Lambris, P. Gros, Structure of C3bfactor H and implications for host protection by complement regulators, Nat. Immunol. 10 (2009) 728–733.

[33] M. Alcorlo, R. Martínez-Barricartea, F.J. Fernández, C. Rodríguez-Gallego, A. Round, M.A. Vega, C.L. Harris, S. Rodríguez de Cordoba, O. Llorca, Unique structure of iC3b resolved at a resolution of 24 Å by 3D-electron microscopy, Proc. Nat. Acad. Sci. U. S. A. 108 (2011) 13236–13240.