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Analysis of a Human Sperm CD52 Glycoform in Primates: Identification of an Animal Model for Immunocontraceptive Vaccine Development[1]

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ABSTRACT

Sperm agglutination antigen-1 (SAGA-1) is a human male reproductive tract glycoform of CD52. Unique modification of CD52 *N*-linked oligosaccharide chains in the epididymis and vas deferens results in the appearance of a carbohydrate epitope that is localized over the entire surface of human spermatozoa. SAGA-1 was characterized by the sperm-inhibitory murine monoclonal antibody (mAb) S19, and it is the target antigen of a human mAb (H6-3C4) associated with antibody-mediated infertility. Collectively, sperm surface localization, antibody inhibition of sperm function, and potential reproductive-tissue specificity identify SAGA-1 as an attractive candidate contraceptive immunogen. To establish an animal model for the study of SAGA-1 in immunologic infertility and immunocontraceptive development, we investigated the appearance of the S19 carbohydrate epitope in nonhuman primates. The S19 mAb demonstrated little to no immunoreactivity by Western blot analysis with protein extracts of spermatozoa from the baboon, marmoset, bonnet, cynomolgus, and pigtailed macaques. Immunohistochemical analysis identified CD52 in the bonnet monkey epididymis; however, the *N*-linked carbohydrate moiety recognized by the S19 mAb, and unique to SAGA-1, was absent. In contrast, the S19 carbohydrate epitope was identified in chimpanzee sperm extracts by Western blot analysis and in chimpanzee epididymal tissue sections by immunohistochemical analysis, indicating that it is conserved in this close relative of the human. Chimpanzee testis, seminal vesicle, and prostate do not express the S19 epitope. Although anti-CD52 immunoreactivity was identified in the spleen, the carbohydrate moiety recognized by the S19 mAb was absent, corroborating data in the human that demonstrated tissue-specific glycosylation of sperm CD52. Immunofluorescent analysis indicated that the chimpanzee homologue of sperm CD52 was present over the entire spermatozoon. In addition, the S19 mAb agglutinated chimpanzee spermatozoa in a manner similar to the effect observed on human spermatozoa. These data indicate that the distinctive carbohydrate moiety of human sperm CD52 is present in the chimpanzee, and they identify the chimpanzee as the most appropriate primate model to study the potential of this unique CD52 glycoform as a contraceptive immunogen.

epididymis, gamete biology, immunology, male reproductive tract, sperm

INTRODUCTION

Identification of sperm antigens capable of eliciting production of functionally relevant sperm antibodies is a first step toward a more complete understanding of the mechanism(s) underlying immunologic infertility. An antisperm immune response is associated with up to 30% of couples presenting with undefined infertility [1]. Immunodominant sperm surface antigens relevant to antibody-mediated infertility, although largely uncharacterized, display considerable heterogeneity [2], perhaps reflecting diversity in the mechanism(s) involved in disruption of the fertilization process. Elucidation of the epitopes targeted by antisperm antibodies would advance the diagnosis of immunologic infertility and provide the basis for immunologic regulation of fertility via a contraceptive vaccine.

Greater than 90% of extracellular membrane proteins in all cell types are glycosylated [3], and the surface of mammalian sperm is no exception, being covered by a dense glycocalyx (for review, see [4]). Monoclonal antibodies directed against sperm surface molecules preferentially target carbohydrate moieties [5, 6]. Moreover, the majority of sperm-immobilizing antibodies in sera from infertile women were directed against carbohydrate epitopes of sperm coating or sperm membrane antigens [7]. These findings reflect the abundance and functional relevance of oligosaccharides that collectively form the sperm glycocalyx. That carbohydrate epitopes are often involved in eliciting antisperm immune responses emphasizes the importance of the chemical makeup of the glycocalyx in terms of understanding and utilizing physiological responses of the immune system during immunocontraceptive vaccine development.

Sperm agglutination antigen-1 (SAGA-1) is a highly acidic (pI 2.5–3), polymorphic (~15–25 kDa) glycoprotein secreted by the epididymis that is localized over the entire human sperm plasma membrane [8]. SAGA-1 was identified as the target of an anticarbohydrate monoclonal antibody (mAb; H6-3C4) immortalized from an infertile woman [9], linking this antigen to the cause of immunologic infertility [10]. An mAb (S19) that displays multiple sperm-inhibitory activities [8, 11, 12] recognizes a unique *N*-linked glycan of SAGA-1 [10]. The peptide core of SAGA-1 is identical to CD52, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein found on the surface of human lymphocytes and spermatozoa. However, S19 immunoreactivity was identified only in the epididymis and on spermatozoa, indicating the S19 carbohydrate moiety was a male reproductive tract-specific epitope [10]. Therefore,

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differential glycosylation of CD52 in the male reproductive tract appears to result in the tissue-specific appearance of an epitope that is the target of sperm-inhibitory mAbs. Collectively, these results identify SAGA-1 as an attractive candidate for development of an immunocontraceptive vaccine. Furthermore, a recombinant single-chain variable region fragment (ScFv) that reacts with SAGA-1 has been engineered and may prove to be effective as a spermistatic agent [13].

To establish a suitable animal model for the study of SAGA-1 in immunologic infertility and contraceptive vaccine development, we examined expression of the S19 carbohydrate epitope in nonhuman primates. Sperm extracts from a New World monkey (NWM; marmoset), Old World monkeys (OWM; bonnet, pigtailed, cynomolgus, and baboon), and a great ape (chimpanzee) were analyzed to determine whether the carbohydrate moiety unique to SAGA-1 was conserved among nonhuman primates. An anti-CD52 mAb, Campath-1M, was employed with the S19 mAb to identify CD52 and potential tissue-specific glycoforms, thereby distinguishing the presence or absence of the C-terminal tripeptide/GPI-anchor of CD52 (the Campath-1M epitope [14]) from differential glycosylation of CD52 among primates. Immunohistochemical analysis of reproductive tissues and CD52-containing spleen tissue sections was conducted to determine whether reproductive tract-specific modification of CD52 occurred in nonhuman primates, as has been established in humans [10, 15]. Finally, functional inhibition of spermatozoa by the S19 mAb identified an appropriate primate model for the study of SAGA-1 in contraceptive vaccine development.

MATERIALS AND METHODS

The University of Virginia Human Research Committee approved all studies involving human semen donors. Informed consent was obtained from each participant in the semen donor program after an explanation of the nature and possible consequences of the studies. All procedures involving the use of animals were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* (National Academy Press, Washington, DC).

Monoclonal Antibodies

The S19 mAb [11] (alternative nomenclature, MHS-8) directed against SAGA-1 was generated by the immunization of mice with human spermatozoa as described previously [8]. Ascites fluid containing the S19 mAb was also generated in mice as described previously [8]. For use in Western blot, immunohistochemical, immunofluorescence, and agglutination analyses, the S19 mAb was isolated by ammonium sulfate precipitation of either ascites fluid or supernatants from in vitro cultures of the MHS-8 hybridoma. The Campath-1M mAb (rat immunoglobulin [Ig] M) was purchased as clarified ascites from Serotec (Raleigh, NC). The H6-3C4 mAb (human IgM), 2B6 (murine IgG_3), 2C6 (murine IgM), and 2E5 (murine IgG_3) mAbs were generous gifts from Dr. Koji Koyama (Hyogo Medical College, Nishinomiyz, Japan).

Preparation of Spermatozoa

Cynomolgus monkey (*Macaca fascicularis*) and pigtailed macaque (*M. nemestrina*) spermatozoa were collected by electro-ejaculation and immediately frozen or handled as described below. Bonnet monkey (*M. radiata*) spermatozoa were generously provided by Dr. A.J. Rao from the Primate Research Laboratory, and marmoset (*Callithrix jacchus*) spermatozoa were a gift from the Wisconsin Regional Primate Research Center. Baboon (*Papio papio*) spermatozoa were obtained from the Southwest Foundation for Biomedical Research (San Antonio, TX). Ejaculated chimpanzee (*Pan troglodyte*) spermatozoa were generously provided by the Coulston Foundation (Alamogordo, NM) and the M.D. Anderson Cancer Center (Bastrop, TX). Human semen, donated by healthy volunteers following 3 days of sexual abstinence, was washed following liquefaction at room temperature, and spermatozoa were either extracted or frozen for future use. Primate spermatozoa from the exudate of freshly collected ejaculates were washed following liquefaction at room temperature. Frozen ejaculates were thawed at 37°C, and spermatozoa were pelleted by centrifugation at 400 $\times$ *g* and washed in Ham F10 medium (Irvine Scientific, Santa Ana, CA). Washed spermatozoa were fixed for immunofluorescent analysis or extracted with 1% (w/v) SDS and centrifuged at 12 000 $\times$ g to remove insoluble debris. Protein concentrations of sperm extracts were determined by BCA protein assay (Pierce Chemical, Rockford, IL) using a BSA standard.

Polyacrylamide Gel Electrophoresis and Western Blot Analysis

Polypeptides were separated by one-dimensional SDS-PAGE using 15% polyacrylamide gels [16] with equal amounts of total protein loaded per lane. Benchmark prestained protein ladder (Gibco-BRL, Grand Island, NY) standards were used to estimate the apparent molecular weight of analyzed proteins. Proteins were electroblotted onto nitrocellulose [17], and membranes were blocked for 30 min at room temperature in PBS containing 0.05% (v/v) Tween-20 (PBS-T) and 5% (w/v) nonfat dry milk. Western blots were incubated with primary antibody diluted in PBS-T/0.5% nonfat dry milk. Primary antibody dilution factors were: S19, 1:10 000; Campath-1M, 1:500; H6-3C4, 2B6, 2C6, and 2E5, 1:100. Blots were washed 3 times with PBS-T and incubated with horse radish peroxidase (HRP)-conjugated goat anti-mouse, anti-rat, or anti-human immunoglobulin (1:5000; Jackson ImmunoResearch Laboratories, West Grove, PA). Secondary antibodies alone served as negative controls. After washing, blots were developed with TMB Membrane Peroxidase Substrate (Kirkegaard & Perry Laboratories [KPL], Gaithersburg, MD).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections from the reproductive tract (testis, epididymis, prostate, seminal vesicle) and spleen of the chimpanzee were a generous gift from the Coulston Foundation. Tissue from the caput, corpus, and cauda epididymis of the bonnet monkey were obtained from the Primate Research Laboratory. Tissues were fixed in neutral-buffered 10% (v/v) formalin (Sigma, St. Louis, MO), and fixed tissues were embedded in paraffin and sectioned (thickness, 5 μm) at the University of Virginia Cell Science Core. Following three 5-min washes in xylene, tissue sections were rehydrated through descending amounts of ethanol following the procedures of Southgate and Trejdosiewicz [18]. Slides were treated with 0.6% (v/v) H_2O_2/methanol for 10 min to abolish endogenous peroxidase activity and washed in distilled H_2O. Slides were then washed in PBS for 10 min, and nonspecific protein-binding sites were blocked by incubation in PBS with 10% (w/v) normal goat serum (NGS). Tissue sections were incubated overnight at 4°C in a humidified chamber with null ascites or the S19 mAb (1:50) or Campath-1M mAb (1:100). Following incubation, tissue sections were washed in PBS and incubated for 1 h at room temperature with HRP-conjugated goat anti-mouse or anti-rat antibodies for the S19 and Campath-1M mAbs, respectively (1:10 000; Jackson ImmunoResearch Laboratories). Negative controls also included secondary antibodies alone. To minimize the possibility of obtaining false-negative results, antigen retrieval was attempted in tissues not displaying immunoreactivity. Tissues were treated with 0.1% (w/v) trypsin in 0.1% $CaCl_2$ (pH 7.8) for 10 min at 37°C, washed in PBS, and blocked with 10% NGS. Immunoreactivity was visualized with the TrueBlue reaction substrate (KPL). Chimpanzee spleen sections were immunostained with TrueBlue and counterstained with a nuclear fast red stain (Contrast Red; KPL). Slides were observed by differential interference contrast (DIC) microscopy, and images were recorded with a Zeiss Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY).

Immunofluorescence Microscopy

Freshly collected chimpanzee spermatozoa were fixed in 2% (w/v) paraformaldehyde in PBS for 20 min, washed in PBS, and air-dried onto poly-L-lysine-coated slides. Nonspecific protein-binding sites were blocked by incubating in PBS with 10% (v/v) NGS for 30 min. Slides were incubated with the S19 (1:100) or Campath-1M (1:100) mAb in PBS/1% NGS for 1.5 h at room temperature. Slides were washed in PBS/1% NGS and incubated for 1.5 h with fluorescein isothiocyanate-conjugated goat anti-mouse IgG or goat anti-rat IgM (Jackson ImmunoResearch Laboratories) for the S19 and Campath-1M mAbs, respectively. Slides were washed in PBS and mounted with SlowFade (Molecular Probes, Eugene, OR). Secondary antibodies alone served as negative controls. Results were

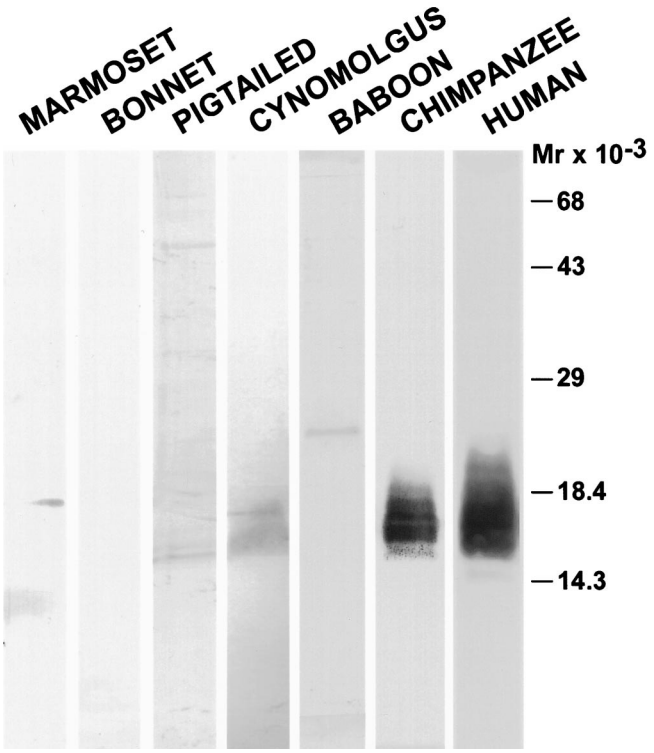

FIG. 1. Identification of the S19 mAb epitope in chimpanzee spermatozoa. A Western blot of sperm extracts (100 μg/lane) from marmoset, bonnet monkey, cynomolgus monkey, pigtailed macaque, baboon, chimpanzee, and human was immunostained with the S19 mAb. A polymorphic antigen of similar molecular mass (~15–25 kDa) was identified in chimpanzee and human spermatozoa.

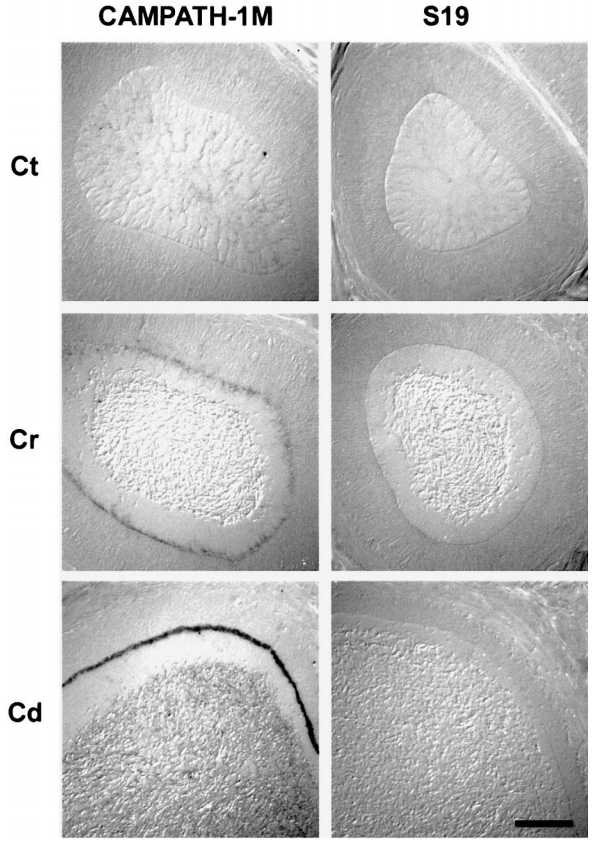

FIG. 2. Immunohistochemical staining of formalin-fixed, paraffin-embedded sections of the caput (Ct), corpus (Cr), and cauda (Cd) epididymis of the bonnet monkey with the Campath-1M or the S19 mAb. Campath-1M immunostained the luminal border, and the intensity of the staining increased distally, with intense immunoreactivity in the cauda epididymis. The S19 mAb failed to stain any region of the bonnet epididymis. Bar = 25 μm.

visualized and recorded with a Zeiss Axiophot microscope (Carl Zeiss) equipped for epifluorescence and DIC microscopy.

Sperm Agglutination Assay

The standard slide agglutination assay was performed as described by Rose et al. [19] and modified by Diekman et al. [8]. Freshly collected chimpanzee spermatozoa were diluted to 20×10^6 cells/ml in Ham F-10 medium and mixed with the S19 mAb purified from ascites fluid (1:5) or with medium alone. Twenty microliters of the sperm solution were placed on a Humagen (Humagen Fertility Diagnostics, Inc., Charlottesville, VA) slide chamber, and sperm agglutination and motility were observed and digitally recorded using DIC optics after 30 min at room temperature.

RESULTS

Evolutionarily Restricted Expression of the S19 Epitope

To examine relative expression of the S19 mAb carbohydrate epitope among primate taxa, spermatozoa were collected from NWM (marmoset), OWM (bonnet monkey, cynomolgus macaque, pigtailed monkey, and baboon), and great ape (chimpanzee), and expression of the S19 epitope was analyzed by Western blot analysis (Fig. 1). The S19 mAb identified a polymorphic (~15–25 kDa) series of immunoreactive bands in chimpanzee sperm extracts that was similar in molecular mass to SAGA-1 from human spermatozoa. In contrast, immunoreactivity corresponding to the S19 carbohydrate moiety was absent from sperm extracts from NWM and most OWM (marmoset, bonnet monkey, pigtailed monkey, and baboon); weak S19 immunoreactivity was detected in cynomolgus sperm extracts. Secondary antibodies alone revealed no immunoreactivity (data not shown). These data suggest that the S19 carbohydrate epitope was conserved in chimpanzees but not in lower-order primates.

Expression of CD52 Is Conserved in the Bonnet Monkey Epididymis

To determine whether the failure to detect the S19 carbohydrate epitope in NWM and OWM could be due to altered expression of CD52 and not differential glycosylation in those species, we examined expression of CD52 in the caput, corpus, and cauda epididymis of the bonnet monkey with Campath-1M, an mAb directed against the C-terminal tripeptide and the GPI-anchor of CD52 [14], and with the S19 mAb. These two antibodies target completely different regions of CD52. Immunoreactivity with the Campath-1M mAb is not dependent on the presence of specific *N*-linked carbohydrate epitopes, but it is dependent on the presence of conserved amino acid residues. Immunostaining with the Campath-1M mAb revealed distinct regional staining of the luminal border of the bonnet monkey epididymis, the intensity of which increased distally, becoming most prominent in the cauda epididymis (Fig. 2). However, the S19 mAb, directed against an *N*-linked carbohydrate epitope, failed to stain any region of the bonnet epididymis. Secondary antibodies alone revealed no immunoreactivity (data not shown).

Identification of the S19 carbohydrate moiety in chimpanzee sperm extracts, but not in those from NWM and

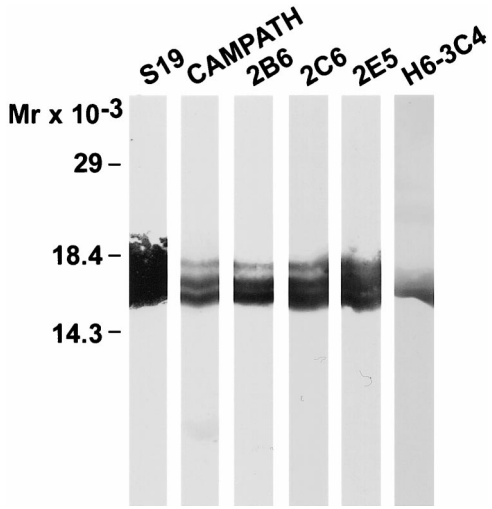

FIG. 3. Western blot of chimpanzee ejaculated sperm extracts (50 μg/lane) immunostained with the S19, Campath-1M, 2B6, 2C6, 2E5, and H6-3C4 mAbs. The Campath-1M, 2B6, 2C6, 2E5, and H6-3C4 antibodies recognized a comparable set of polymorphic bands migrating between approximately 18 and 22 kDa, slightly less heterogeneous in apparent molecular mass compared to immunostaining with the S19 mAb.

OWM, despite identification of CD52 expression in the cynomolgus monkey [20] and bonnet monkey, led us to analyze in more detail the structural conservation of chimpanzee CD52 with antibodies directed against portions of its *N*-linked glycan or C-terminal tripeptide/GPI-anchor. In addition to the S19 mAb, the Campath-1M and four other mAbs (2B6, 2C6, 2E5, and H6-3C4) known to recognize carbohydrate epitopes on CD52 were used to immunostain Western blots of chimpanzee sperm extracts (Fig. 3). Results indicated that each mAb recognized a comparable set of polymorphic bands in chimpanzee sperm extracts that migrated between approximately 18 and 22 kDa, which is slightly less heterogeneous in apparent molecular mass compared to immunostaining with the S19 mAb. Secondary antibodies alone revealed no immunoreactivity (data not shown).

Expression of the S19 Epitope Is Restricted to the Epididymis

Because an important criterion for a sperm-based contraceptive immunogen is tissue specificity, we investigated expression of the S19 mAb carbohydrate epitope in various chimpanzee reproductive tissues. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded testis, seminal vesicle, prostate, and epididymal tissue sections using the S19 mAb and HRP-conjugated anti-mouse Ig (Fig. 4). Immunostaining revealed expression of the S19 mAb epitope in the epididymis but not in the testis, seminal vesicle, or prostate tissue sections (Fig. 4, A–D). In the chimpanzee epididymis, S19 immunoreactivity was identified at the apical aspect of principal cells lining the lumen as well as on spermatozoa within the lumen, similar to the expression pattern observed in the human epididymis [10]. A similar pattern of immunoreactivity was observed in the chimpanzee epididymis using the Campath-1M mAb (data not shown). Tissue sections incubated with null ascites revealed no immunoreactivity (Fig. 4, E–H).

To investigate whether the carbohydrate moiety observed in the chimpanzee epididymis represented a tissue-specific glycoform of CD52, formalin-fixed, paraffin-embedded chimpanzee spleen tissue sections were incubated with the anti-CD52 mAb Campath-1M or the S19 mAb, immunostained with TrueBlue, and counterstained with Contrast Red. The T cells at the periphery of the follicle were immunostained by the Campath-1M mAb; however, the germinal center, containing predominantly B cells, demonstrated little to no immunoreactivity (Fig. 5A). No immunoreactivity was visible in the perifollicular zone or in the germinal center of spleen sections incubated with the S19 mAb (Fig. 5B) or with secondary antibodies alone (Fig. 5, C and D), indicating that the S19 mAb epitope identified on chimpanzee sperm CD52 was not expressed in the chimpanzee spleen. Therefore, the S19 carbohydrate epitope may represent a male reproductive tract-specific product.

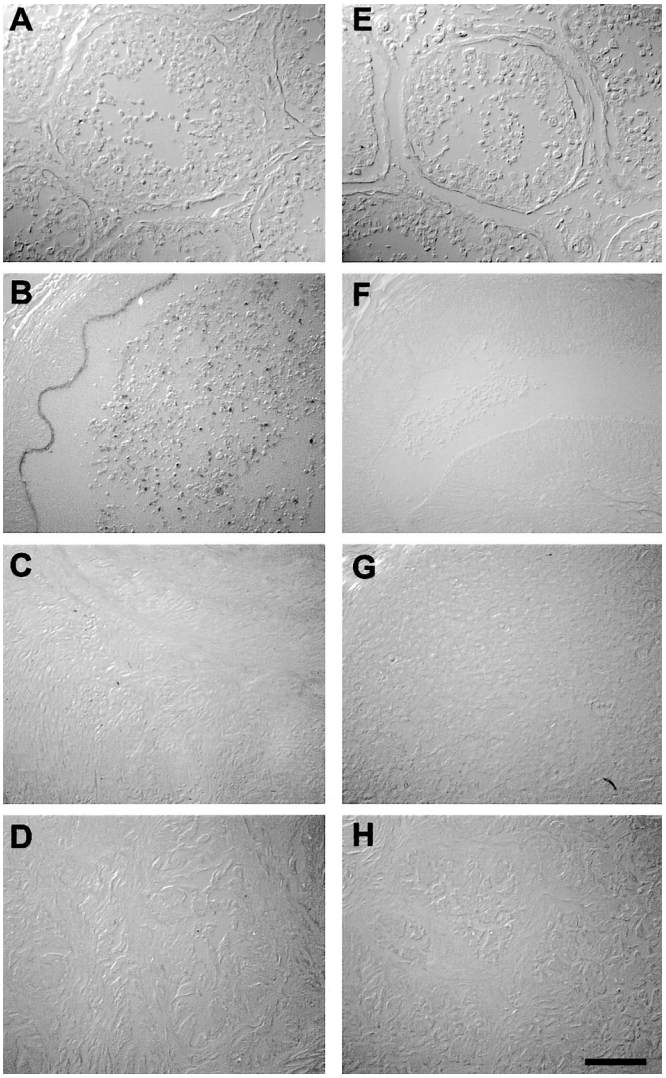

FIG. 4. Immunohistochemical staining of formalin-fixed, paraffin-embedded chimpanzee testis (**A** and **E**), epididymis (**B** and **F**), seminal vesicle (**C** and **G**), and prostate (**D** and **H**) with the S19 mAb (**A**–**D**) or null ascites (**E**–**H**). In the epididymis, immunoreactivity with the S19 mAb was observed at the apical aspect of epithelial cells lining the lumen as well as on spermatozoa within the lumen. Treatment with null ascites revealed no immunoreactivity. Bar = 50 μm.

Localization of the S19 Epitope and Functional Inhibition of Chimpanzee Spermatozoa

Indirect immunofluorescent analysis was performed on paraformaldehyde-fixed chimpanzee sperm to determine

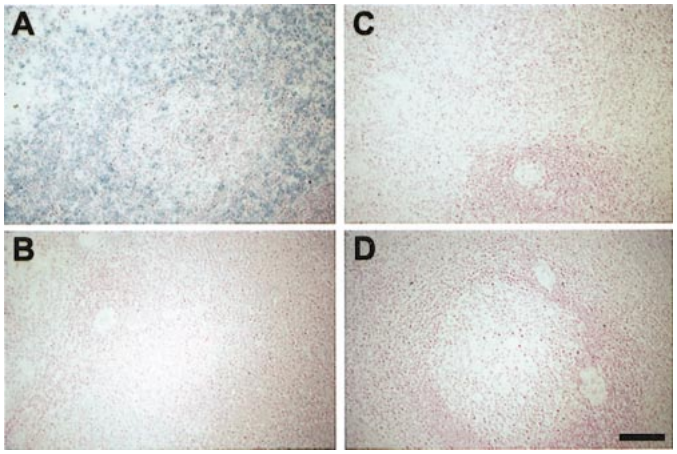

FIG. 5. The S19 epitope is absent from CD52 in chimpanzee lymphoid organs. Formalin-fixed, paraffin-embedded chimpanzee spleen tissue sections were immunostained with the Campath-1M (**A**) or S19 mAb (**B**) and counterstained with a nuclear fast red stain (Contrast Red). The T cells at the periphery of the follicle were immunostained by the Campath-1M mAb; however, the germinal center, containing predominantly B cells, demonstrated little staining. No immunoreactivity was visible with the S19 mAb. Secondary antibody controls for the Campath-1M and S19 mAbs (**C** and **D**, respectively) showed no immunoreactivity. Bar = 10 μm.

whether the S19 epitope was localized on the entire chimpanzee sperm surface, as it is in the human [8]. Similar to the human, both the S19 and Campath-1M mAbs bound to chimpanzee sperm, with fluorescence localized over the entire cell (Fig. 6, A and B). Secondary antibodies alone revealed no staining (Fig. 6, C–F).

To assess the inhibitory activity of the S19 mAb on chimpanzee spermatozoa, agglutination analysis was performed with freshly collected chimpanzee semen. Semen was diluted in Ham F10 media and mixed with an equal volume of medium alone (Fig. 7A) or the S19 mAb (Fig. 7B) and then incubated at room temperature. Agglutination was observed as the cross-linking or clumping together of spermatozoa only in the S19 mAb-treated sample, indicating that the carbohydrate moiety is present on the surface of chimpanzee spermatozoa and is accessible to functional inhibition by the S19 mAb.

DISCUSSION

This comparative study of expression of CD52 and a unique *N*-linked epitope on CD52, SAGA-1, using semen from NWM, OWM, and a great ape indicated that a unique carbohydrate moiety of human CD52 was conserved in the chimpanzee. Little or no immunoreactivity corresponding to the carbohydrate epitope was apparent in NWM or OWM. Western blot analysis performed with a panel of previously characterized mAbs known to recognize oligosaccharide epitopes on human CD52 indicated that the *N*-linked glycan, including the S19 epitope present on a human sperm-specific glycoform of CD52, was highly con-

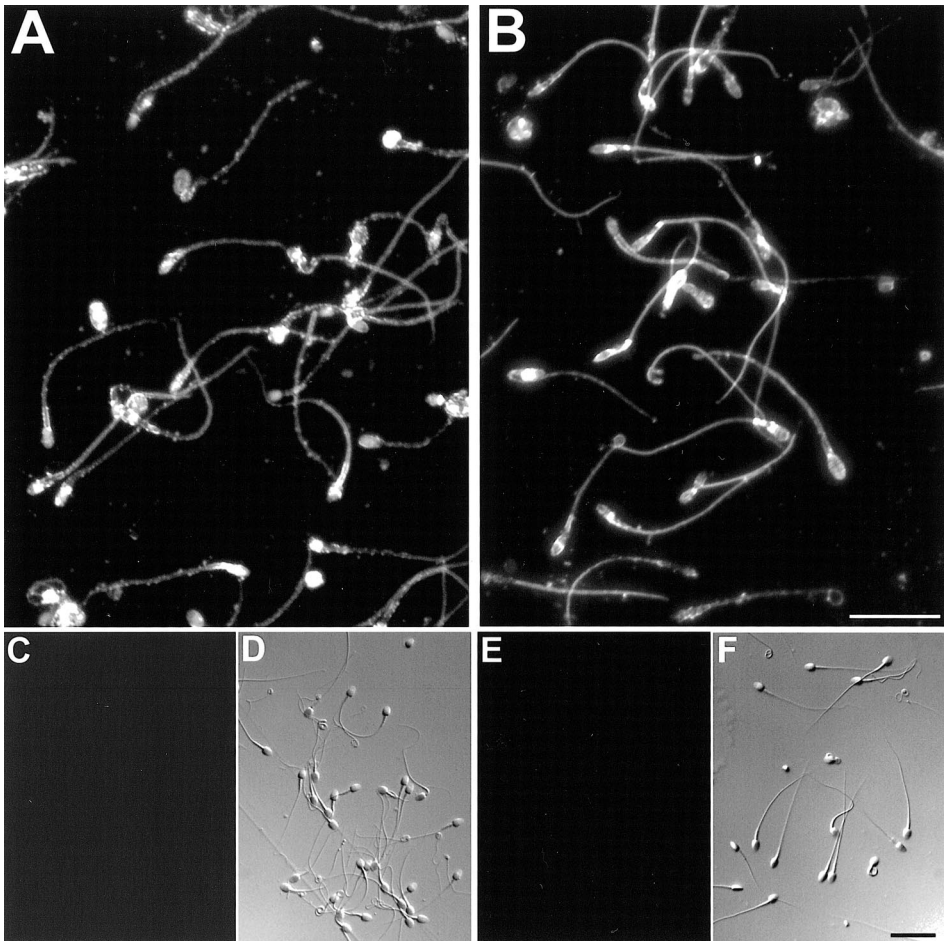

FIG. 6. Indirect immunofluorescent staining of paraformaldehyde-fixed chimpanzee spermatozoa with the mouse S19 mAb (**A**) and the rat Campath-1M mAb (**B**). Immunofluorescence was observed over the entire surface of chimpanzee sperm stained with both antibodies. No immunofluorescent staining was observed with secondary anti-mouse (**C** and **D**) and anti-rat (**E** and **F**) antibody-negative controls. Both immunofluorescence (**A**–**C** and **E**) and DIC microscopy (**D** and **F**) are shown. Bar = 10 μm.

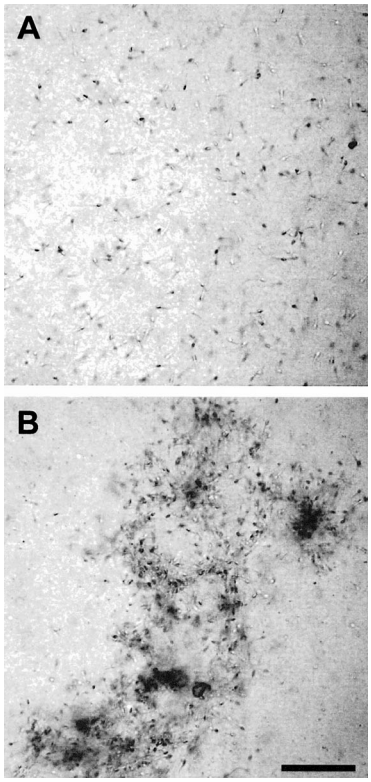

FIG. 7. Functional inhibition of chimpanzee spermatozoa by the S19 mAb. Live chimpanzee spermatozoa were diluted in Ham F10 media and mixed with an equal volume of medium alone (**A**) or the S19 mAb (**B**), and sperm motility was observed. Agglutination in a tangled pattern was observed only in the sample treated with the S19 mAb. Bar = 100 μm.

served in chimpanzee spermatozoa. The S19 epitope has been localized over the entire human sperm surface by immunofluorescence and ultrastructural analysis [21]. In the present study, the S19 epitope localized to the entire surface of chimpanzee spermatozoa, rendering cells susceptible to agglutination on exposure to the anti-SAGA-1 S19 mAb. Investigation of the apparent hominid-specific expression of the S19 epitope identified differential expression of the Campath-1M and S19 epitopes in the bonnet epididymis. Although CD52 was expressed in the epididymis of the bonnet monkey, the glycoform of CD52 recognized by the S19 mAb was not detected in parallel tissue sections or on Western blots of bonnet sperm extracts. Therefore, despite conservation of the C-terminal tripeptide/GPI-anchor of CD52, the unique glycosylation of CD52 with concomitant expression of the S19 mAb carbohydrate epitope that is apparent in humans and chimpanzees was not conserved in the OWM. Collectively, these results indicated that the carbohydrate epitope recognized by the S19 mAb either was not as abundant or was not structurally conserved in NWM or OWM relative to the chimpanzee or human.

In the chimpanzee, expression of the S19 epitope was identified in the epididymis but not in the testis, seminal vesicles, or prostate, similar to immunohistochemical and Western blot data from the human (unpublished data). Epididymal localization of the S19 mAb epitope in chimpanzees was identical to localization in the human epididymis [10]. The S19 immunoreactivity was identified on the microvilli of epithelial cells surrounding the epididymal lumen and on spermatozoa in the lumen. In addition, immunohistochemical analysis of expression of CD52 and its unique *N*-linked glycan demonstrated that the carbohydrate epitope recognized by the S19 mAb was not present in the chimpanzee spleen despite expression of CD52. Combined with the finding that S19 epitope was expressed in the epididymis but not in other male reproductive tissues tested, these data indicate that a tissue-specific CD52 glycoform is expressed in the chimpanzee, and they corroborate data in the human that identified a CD52 glycoform [10] that appears to be a male reproductive tract-specific product.

To evaluate the potential use of an antigen as a contraceptive agent, it is preferable to identify a nonhuman primate model system possessing a homologue of the given antigen. Although a number of mammalian homologues of CD52 are known [22, 23], these sequences are similar only in their signal sequences and not in the core peptide. Moreover, the present study highlights the importance of posttranslational modification of sperm surface CD52 with respect to identification of a nonhuman primate model expressing a homologue to this defined glycoprotein. CD52 was identified in the bonnet epididymis, but the CD52 glycoform recognized by the S19 mAb was not detected. The S19 immunoreactivity was not present in NWM or OWM but was identified in the chimpanzee epididymis and on chimpanzee spermatozoa.

Carbohydrate moieties on sperm surface glycoproteins may be added or exposed during sperm maturation [24–27] and/or capacitation [28]. Glycosylated GPI-anchored sperm proteins such as CD59 [29], CD55 [30], and CD52 [28, 31, 32] are secreted by the epididymal epithelium and inserted into the sperm membrane during epididymal transit [33, 34]. The S19 carbohydrate epitope is acquired on the sperm surface during transit of spermatozoa through the epididymis, and a concomitant increase in heterogeneity of S19 immunoreactivity accompanies sperm migration through the caput, corpus, and cauda epididymidal regions (unpublished data). The regulation of cell-specific and tissue-specific glycans has been attributed to the presence and activity of glycosyltransferases (for review, see [35]), many of which display unique tissue distribution and substrate specificity [36]. Although only speculative at this time, identification of an apparently tissue-specific S19 carbohydrate epitope on CD52 in chimpanzees and humans suggests that a unique glycosyltransferase either exists in the epithelium or lumen of the hominid epididymis or is uniquely regulated by the epididymal environment. Alternatively, exposure to glycosidase or protease activity may contribute to appearance of the S19 epitope. Although epididymal epithelial cell culture has been moderately successful in a variety of species [37–40], including the human [41], development of an improved, more stable epididymal cell culture system would enhance efforts directed at the identification of glycosyltransferase activity potentially responsible for expression of the unique sperm surface S19 epitope.

Schröter et al. [15] demonstrated that more than 50 different *N*-linked oligosaccharide structures were present in human seminal plasma CD52, a much higher degree of heterogeneity compared to the carbohydrate structures identified in spleen CD52 [42]. These data underscore the need to characterize carbohydrate epitopes potentially viewed as targets for immunocontraceptive development. Target epitopes for immunocontraceptive development should be sperm-specific to prevent harmful cross-reactivity with somatic tissues. For example, unlike the anticarbohydrate 2B6, 2C6, and 2E5 mAbs, the S19 mAb epitope appears to be specific to a sperm glycoform of human CD52 [10], diminishing possible concerns regarding induction of au-

toimmune disease in SAGA-1 immunogenicity studies due to cross-reactive somatic tissues.

The principal finding of the present study is that a unique glycoform of CD52 is structurally conserved in chimpanzees but not in lower-order nonhuman primates, identifying the chimpanzee as an appropriate nonhuman primate model to advance immunocontraceptive vaccine development of SAGA-1. Of particular interest is that humans are most closely related to the chimpanzee, sharing approximately 99% genetic identity [43–46]. Thus, it is reasonable to speculate that an evolutionary event may have occurred after divergence of hominids from the OWM some 31 million years ago [46] that led to the expression of a unique carbohydrate epitope in the epididymis. That such an evolutionary event could have altered the structure of the *N*-linked glycan of CD52 is supported by the example of loss of expression in humans of a carbohydrate epitope (*N*-glycolylneuraminic acid) common in all other mammals, including the great apes [47]. One would expect to find few genetic differences between chimpanzees and humans, yet a mutation in human cytidine monophospho-sialic acid hydroxylase led to the lack of expression in humans of a common type of sialic acid found in chimpanzees. Accelerated evolution of male reproductive genes has been noted in primates and in the lineages leading to both the chimpanzee and human [48]; thus, the expression of a carbohydrate epitope on spermatozoa from chimpanzees and humans, but not from other primates, may be linked to an evolutionary change related to glycan synthesis. Because of the lack of conserved expression in other primates, the most informative preclinical studies regarding the immunogenic and contraceptive potential of SAGA-1 would derive from investigations using the chimpanzee primate model.

To our knowledge, differences in sperm surface carbohydrates have not been previously demonstrated among primates. As more knowledge is gained regarding the role of such carbohydrates in sperm function, it will be important to test if such differences relate to primate speciation.

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