Identification of highly conserved and species-specific polypeptides of *Haemophilus ducreyi*

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Summary. Chancroid is a sexually transmitted disease caused by *Haemophilus ducreyi*. The pathological manifestations of chancroid are unique among *Haemophilus* species and the virulence factors of *H. ducreyi* that account for these features have not been identified. Some of these virulence factors may be unique components of *H. ducreyi*, but attempts to identify *H. ducreyi*-specific components have been unsuccessful. Four polypeptides—A, B, C and D of 83, 77, 56 and 28 kDa, respectively—were identified with a panel of nine *H. ducreyi*-specific monoclonal antibodies (MAbs). Polypeptide C was one of the five major proteins in *H. ducreyi* and demonstrated micro-heterogeneity in SDS-PAGE. Polypeptides A, B and D were present in only small amounts in whole-cell lysates of *H. ducreyi*. The relative amounts of A and B varied, suggesting that they may be precursor molecules. The unique polypeptides C and D were not exposed on the surface. Polypeptide C was highly soluble and did not appear to be membrane-bound, whereas polypeptide D appeared to partition with the cytoplasmic membrane and was soluble in Sarkosyl. All four polypeptides appeared to be unique to *H. ducreyi* since MAbs directed against them did not cross-react with *H. influenzae*, *H. parainfluenzae* or *Neisseria gonorrhoeae*. The mol. wts of all of these polypeptides were conserved throughout 35 clinical isolates collected from 15 cities in eight countries and one reference strain of *H. ducreyi* that were tested. Based on the immune cross-reactivities, it was concluded that the four polypeptides belonged to a family of proteins with partial sequence homology that may have been derived from the same precursor polypeptide or the same transcription unit. A sequence relationship model for the polypeptides was proposed based on their cross-reactivities with the MAbs.

Introduction

Haemophilus ducreyi is the aetiological agent of chancroid, a sexually transmitted disease that is characterised by genital ulcers and painful, swollen, regional lymph nodes that may suppurate to form “buboes”. Although *H. ducreyi* does not spread systemically, the virulence factors that allow it to evade the host defence mechanisms and localise in the regional lymph nodes are as yet unknown. However, the pathological manifestations of infection with *H. ducreyi* are very different from those of diseases caused by other *Haemophilus* species.^{1,2} It is expected that at least some of the virulence factors of *H. ducreyi* must be unique to this micro-organism. Attempts to identify those unique components have not been extensive and have been largely unsuccessful because of the extensive immune cross-reactivity of *H. ducreyi*

with other *Haemophilus* spp. Injection of *H. ducreyi* into rabbits results in a strong humoral response, but the antisera also cross-react strongly with *H. influenzae* and *H. parainfluenzae*.^{3–5} This high degree of cross-reactivity has contributed to the difficulty of identifying those components that are unique to *H. ducreyi*. Odumeru *et al.*⁶ demonstrated that c. 50 % of the anti-*H. ducreyi* antibodies produced in rabbits or mice cross-reacted with antigens of *H. influenzae* or *H. parainfluenzae*. Indeed, Hansen and Loftus⁷ and Schalla *et al.*⁸ have attempted to develop monoclonal antibodies (MAbs) specific to *H. ducreyi*. The antigens recognised by these MAbs have not been fully characterised. Because of these problems, no *H. ducreyi*-specific polypeptides have been identified and little is known about *H. ducreyi*-specific components.

Based on a competition-adsorption method for hybridoma screening, a panel of nine independently isolated hybridoma clones producing *H. ducreyi*-specific MAbs was produced.⁶ This report describes the identification and characterisation of four polypeptides that are recognised by the *H. ducreyi*-specific

MAbs and their localisation and conservation among clinical isolates.

Materials and methods

Bacterial stock cultures

The strains of *H. ducreyi* used in this study were clinical isolates and represented strains collected from around the world (table). They were maintained in skimmed milk at -70°C until required. The *H. ducreyi* strains were propagated on chocolate blood agar supplemented with Isovitalex (BBL) 1% and horse serum 5% v/v at 34°C in high humidity and CO_2 5% in air.

Monoclonal antibodies

The production and initial characterisation of the panel of nine *H. ducreyi* MAbs was described by Odumeru *et al.*⁶ In this study, ascites fluid was produced by injecting 1×10^6 hybridoma cells intraperitoneally into BALB/c mice (University of

Manitoba Central Animal Unit) that had been primed 7 days earlier with an intraperitoneal injection of 0.5 ml of pristane. Cells and debris were removed by centrifugation at $6000g$ for 10 min and the ascites fluid was then stored at -20°C until required.

SDS-PAGE and Western immunoblotting procedures

H. ducreyi 409 cells from 48-h cultures were solubilised in sample buffer (62.5 mM Tris-HCl, pH 6.5; SDS 2% w/v, 2-mercaptoethanol 5% v/v, glycerol 10% v/v, bromophenol blue 0.01% w/v) and heated at 98°C for 5 min. The protein bands were separated by electrophoresis on a discontinuous gel⁹ consisting of acrylamide 12.5% and bis-acrylamide 0.5%. The gel was either stained with Coomassie Blue or transferred electrophoretically to nitrocellulose (0.22 μm pore diameter, Schleicher and Schull) at 200 mA for 4 h. The immunoblotting buffer consisted of 25 mM Tris base (BioRad Laboratories), 200 mM glycine (BioRad Laboratories) and methanol 20% v/v. Residual protein binding sites were blocked by incubating the nitrocellulose in bovine serum albumin (BSA; Sigma) 3% w/v in Tris-buffered saline (TBS; 20 mM Tris-HCl, 500 mM NaCl, pH 7.5) at room temperature for 4 h. The nitrocellulose was then incubated with the appropriate MAb diluted 1 in 100 in BSA-TTBS (BSA 1% w/v, Tween 20 0.05% v/v in TBS) at 4°C for 18 h. After washing the nitrocellulose three times for 30 min each with TTBS to remove unbound MAb, the nitrocellulose was incubated for 2 h at room temperature with peroxidase-conjugated goat anti-mouse IgG, IgM, IgA (Cedar Lane) diluted 1 in 1000 in BSA-TTBS. The nitrocellulose was then washed three times for 30 min with TTBS to remove unbound peroxidase conjugate. Colour was developed with 4.4 chloro-naphthol (BioRad) as described by the manufacturer.

¹²⁵I-Iodination of cell-surface components

The method of Fraker and Speck¹⁰ was used to iodinate the outer-membrane proteins (OMPs) of *H. ducreyi*. Briefly, a whole-cell suspension of a 24-h culture of *H. ducreyi* was washed once with PBS. Clumps were allowed to settle and the homogeneous portion of the cell suspension (50 μl) was pipetted into a glass vial that had been coated previously with 1 μg of Iodagen (Pierce Chemical Co.). Carrier-free ¹²⁵I (0.5 μCi) was added and the reaction was allowed to proceed for 15 min. The *H. ducreyi* suspension was removed from the reaction tube and washed three times with 200 μl of PBS to remove unbound ¹²⁵I. The trichloroacetic acid precipitability of such labelled preparations was $> 95\%$. The ¹²⁵I-labelled *H. ducreyi* cells were solubilised in sample buffer, heated at 98°C for 5 min and then stored at -20°C until required.

Subcellular fractionation

H. ducreyi cells were suspended in TBS and disrupted at -20°C in an X-press (Fred S. Carver Inc.,

Table. *H. ducreyi* strains*

Sample no.	Strain designation	Source
1	35000	Winnipeg, MB, Canada
2	35199	Winnipeg, MB, Canada
3	54198	Winnipeg, MB, Canada
4	78226	Winnipeg, MB, Canada
5	CIP542	Paris, France
6	GV072	Bathurst, Gambia
7	GV073	Bathurst, Gambia
8	181	Nairobi, Kenya
9	409†	Nairobi, Kenya
10	9468	Nairobi, Kenya
11	AX557	Nairobi, Kenya
12	BG407	Nairobi, Kenya
13	BG411	Nairobi, Kenya
14	C13	Nairobi, Kenya
15	C143	Nairobi, Kenya
16	HD013	Amsterdam, Netherlands
17	HD014	Amsterdam, Netherlands
18	E1673	Stockholm, Sweden
19	HD1	Stockholm, Sweden
20	CH2	Bangkok, Thailand
21	CH39	Bangkok, Thailand
22	PU1	Bangkok, Thailand
23	CA173	California, USA
24	WPB506	W. Palm Beach, CA, USA
25	WPB511	W. Palm Beach, CA, USA
26	6V	Atlanta, GA, USA
27	108	Atlanta, GA, USA
28	OR574	Orlando, FL, USA
29	MASS576	Boston, MA, USA
30	MASS579	Boston, MA, USA
31	NY006	New York, NY, USA
32	NY012	New York, NY, USA
33	OH1075	Ohio, USA
34	VII58	Seattle, WA, USA
35	VII59	Seattle, WA, USA
36	36-F-2	Reference strain

*These bacterial strains were derived from A. R. Ronald's collection of *H. ducreyi* isolates that were originally isolated in the countries indicated.

†Strain 409 was used for the immunisation of mice in the production of the MAbs⁶ and was used in the present study.

NJ, USA). The cell debris was collected by centrifugation at 10000 *g* for 20 min (P-10). The supernate (S-10) was further fractionated by centrifugation at 100000 *g* for 60 min. The high speed supernate (S-100) was collected. The pellet (P-100) was extracted with sodium lauryl sarcosinate (Sarkosyl) 2% (w/v) in 10 mM HEPES, pH 7.4, and separated by centrifugation at 100000 *g* for 60 min into the Sarkosyl extract supernate (SS-100) and the pellet containing the detergent-insoluble outer-membrane fraction (OMP^{11,12}). The final volume at each stage was adjusted to maintain equivalent relative volume (1 ml). A sample of each cell fraction was saved for quantitative comparison by SDS-PAGE.

Results

The proteins from *H. ducreyi* cells lysed in SDS, separated by SDS-PAGE and blotted on to nitrocellulose were probed with the nine MABs that reacted specifically with *H. ducreyi* and did not cross-react with other *Haemophilus* species⁶ (fig. 1). Four *H. ducreyi* polypeptides, A, B, C and D, of 83, 77, 56 and 28 kDa, respectively, were recognised by the nine MABs in the panel (fig. 1). MABs MHd083 and

Fig. 1. Identification of *H. ducreyi* polypeptides recognised by the *H. ducreyi*-specific MABs. Whole-cell preparations of *H. ducreyi* 409 (7 µg/lane) were solubilised in sample buffer, resolved by SDS-PAGE and electroblotted on to nitrocellulose. The nitrocellulose strips were separately probed with MABs MHd083 (lane a), MHd084 (b), MHd078 (c), MHd080 (d), MHd072 (e), MHd107 (f), MHd091 (g), MHd082 (h) and MHd086 (i). The migration of biotinylated mol.-wt markers (kDa) phosphorylase B (92.5), bovine serum albumin (66.2), ovalbumin (45), carbonic anhydrase (31), soybean trypsin inhibitor (21.5) and lysozyme (14.4) are indicated.

MHd084 were specific for polypeptide D (fig. 1, lanes a and b), and MAB MHd086 reacted only with polypeptide C (fig. 1, lane i). However, each of the other MABs reacted with multiple polypeptides. MAB MHd078 reacted with polypeptides A, B and D (fig. 1, lane c) and MAB MHd072 recognised polypeptides B, C and D (fig. 1, lane e). Both MABs MHd082 and MHd091 bound to polypeptides B and C (fig. 1, lanes g and h), although the reactivity of MAB MHd082 with polypeptide C (fig. 1, lane h) was very weak. The similar reactivity of MABs MHd082 and MHd091 suggests binding to a similar region but the relative reactivity of binding indicates that they may bind different epitopes. MAB MHd107 (fig. 1, lane f) appeared to have specificity similar to that of MAB MHd072 (fig. 1, lane e) but its reactivity with polypeptides B and C was very weak. Although polypeptide A was detected in all strains tested, the relative concentration of polypeptide A varied from one preparation to another, suggesting that it may be an unstable pre-protein. The immune cross-reactivity indicated an antigenic relationship between polypeptides A, B, C and D (fig. 1).

The presence of these *H. ducreyi*-specific polypeptides was tested by Western immunoblotting against 35 isolates of *H. ducreyi* collected from eight countries around the world and one reference strain, 36-F-2. Because of the variability in the relative concentration of polypeptide A, only polypeptides B, C and D were studied further. MAB MHd072 was used to assess the presence of polypeptides B and D and MAB MHd091 was used to assess the presence of polypeptide C. As shown in fig. 2, not only was an antigenic analogue of each of polypeptides B, C and D detected in all the isolates tested but also the apparent mol. wts of the polypeptides were conserved, as determined by immunoblot after SDS-PAGE, in all of the 36 *H. ducreyi* strains tested. Therefore, these antigens were not only unique to *H. ducreyi* but also appeared to be ubiquitous within the species with extremely well conserved mol. wts among all the *H. ducreyi* strains. No other *H. ducreyi*-specific polypeptide that was not related to this family of polypeptides was recognised by the panel of MABs studied.

To determine whether any of these polypeptides was exposed on the cell surface, *H. ducreyi* whole cells were surface ¹²⁵I-iodinated, washed and solubilised in SDS. The proteins were separated by SDS-PAGE and then the ¹²⁵I-labelled bands were detected by autoradiography (fig. 3A, lane 9). The 31-kDa polypeptide was predominantly radiolabelled followed by the 40-, 44- and 47-kDa protein bands. None of these radiolabelled bands corresponded to the *H. ducreyi*-specific polypeptide C detected with MAB MHd086 in an immunoblot (fig. 3B, lane 8), or polypeptides B and D based on their apparent mol. wts (fig. 1).

To determine whether any of these polypeptides could be a component of the outer membrane (OM) which cannot be labelled by our surface iodination method, the OM was isolated by the Sarkosyl ex-

Fig. 2. Detection of epitopes bound by *H. ducreyi*-specific MAbs in different *H. ducreyi* isolates. Cells of individual *H. ducreyi* isolates were lysed in sample buffer at 95°C, separated by SDS-PAGE and blotted on to nitrocellulose. For immunoblot detection of polypeptide C (C) with MHd091, 1 µg of cell protein/lane was analysed; for the detection of polypeptides B (B) and D (D) with MHd072, 10 µg/lane was loaded on the gel. The lane designations for individual bacterial strains tested are provided in the table. Only the relevant areas of the immunoblots showing the polypeptides are shown in the composite figure.

Fig. 3. Subcellular localisation of *H. ducreyi*-specific polypeptides. Whole cells of *H. ducreyi* 409 (lane 1) were lysed by X-press and clarified by centrifugation at 10000 *g* to remove cell debris (P-10; lane 2). The supernate (S-10; lane 3) was further fractionated by centrifugation at 100000 *g* into the high speed pellet (P-100; lane 4) and high speed supernate (S-100; lane 5), respectively. The pellet was resuspended and extracted with Sarkosyl 2% and centrifuged at 100000 *g* for 60 min to separate the detergent-insoluble outer-membrane preparation (OMP; lane 6) from the Sarkosyl extract (SS-100; lane 7). The final relative volume of each fraction (1 ml) was maintained constant for final analyses (10 µl/lane) by SDS-PAGE, visualised by Coomassie Brilliant Blue (Panel A), and immunoblots, with MAb MHd086 to detect polypeptide C (panel B) and MAb MHd084 to detect polypeptide D (panel C). The presence of polypeptide B in the OMP fraction was tested for with MAb MHd082 (panel C, lane 9). Whole cells that had been surface ¹²⁵I-labelled were analysed by SDS-PAGE and the labelled polypeptide bands were visualised by autoradiography (panel A, lane 9) and immunoblotted for polypeptide C (B, lane 9) and immunoblotted for polypeptide D (C, lane 9). The Coomassie Blue-stained mol.-wt markers (lane A8) and biotinylated mol.-wt markers (lane B8, C8) were similar to those described in fig. 1.

traction method and separated by SDS-PAGE (fig. 3A, lane 6). The OMP profile (fig. 3A, lane 6) was similar to the surface-iodinated cell-protein profile

detected by autoradiography (fig. 3A, lane 9). However, none of the protein bands in the OMP fraction co-migrated with polypeptides B, C and D (fig. 1) and

polypeptide C (fig. 3B, lane 6), B or D (fig. 3C, lane 6, 9) were not detected in the Sarkosyl-insoluble OMP fraction by immunoblotting with MAbs MHd086, 082 and 084, respectively. Polypeptides B, C and D must not be part of the OMP structure. Although all these polypeptides were found in the soluble fraction, the subcellular distribution of polypeptide D was slightly different from that of polypeptides B and C. As shown in fig. 3B, polypeptide C segregated into the S-10 and S-100 fractions (fig. 3B, lanes 3, 5) but not in the P-10 or P-100 fractions (fig. 3B, lanes 2 and 4), indicating that it is a soluble protein which is likely to be located in the cytosol or the periplasmic space. However, the detection of polypeptide D in the P-10 and P-100 fractions (fig. 3C, lanes 2 and 4), in addition to its presence in the S-10, S-100 and SS-100 (fig. 3C, lanes 3, 5 and 7), but not in the Sarkosyl-insoluble OMP fraction (fig. 3C, lane 6) indicated that a fraction of polypeptide D may be associated with the cytoplasmic membrane.

Discussion

The nine independently isolated MAbs specific for *H. ducreyi*⁶ recognised only four separate polypeptides designated A, B, C and D (fig. 1) of 83, 77, 56 and 28 kDa, respectively. The epitopes bound by the MAbs on these four polypeptides were unique to *H. ducreyi* since the MAbs did not cross-react with whole cells or cell lysates of different strains of *H. influenzae*, *H. parainfluenzae* or *N. gonorrhoeae*,⁶ or *Treponema pallidum* (Nichols strain), *Escherichia coli* 392 or *Staphylococcus aureus* (Cowan strain). The antigens recognised by these *H. ducreyi*-specific MAbs appeared to be distinct from that recognised by the MAb 10-14-kDa produced by Hansen and Loftus.⁷ The 46-, 41- and 28-kDa polypeptides described by Abeck *et al.*¹¹ that reacted with immune mouse sera correlate with the surface-labelled OMPs reported here (fig. 3A, lane 9) as well those described by Abeck and Johnson.¹²

Polypeptide C was one of the most abundant proteins in the whole-cell profile of *H. ducreyi* whereas polypeptide D was a minor component and polypeptides A and B could barely be detected by Coomassie Blue staining (fig. 3A, lane 1). The immune cross-reactivities of the MAbs with the four polypeptides indicated that the latter must belong to a family of polypeptides with varying extent of sequence homologies. Furthermore, polypeptide A, which has the largest mol. wt and exhibits variable concentration in the cell, may be a pre-protein for some of the small and more stable members of the family.

Unlike OMP profiles, that can vary significantly between different strains,^{13,14} polypeptides B, C and D were conserved in all 36 isolates of *H. ducreyi* collected from 15 cities in eight countries over a span of more than 40 years (36-F-2, Pittman strain, 1943). Not only were antigenic analogues of each of these polypeptides

detected in Western blots with MAbs, but also the mol. wts of each of these polypeptides were completely conserved among all clinical isolates tested. The extent of conservation in the amino-acid sequences and nucleotide sequences must await the purification or molecular cloning of these polypeptides. However, based on the obligatory expression and conservation of the mol. wts of these *H. ducreyi*-unique polypeptides, it is likely that these polypeptides may have an important role in the biology of the organism or the pathogenesis of chancroid, or both, in order to be selected against random mutation as seen in many cellular proteins, including the OMPs.^{11,15}

To determine whether these bacterial components are presented on the cell surface for interaction with the host, *H. ducreyi* whole cells were surface-labelled with ¹²⁵I by the Iodagen method. Iodagen is water-insoluble and, once coated on to the glass surface of the reaction tube, very little can become detached in aqueous solution to penetrate the cell during iodination.¹⁰ As a result, predominantly surface-exposed components are labelled. SDS-PAGE analysis of surface-iodinated *H. ducreyi* strain 409 indicated that there were four surface-exposed polypeptides of 46, 44, 41 and 31 kDa, respectively (fig. 3A, lane 9). The 31-kDa component was the most heavily radiolabelled. This is consistent with the observation of Abeck and Johnson¹² that the 27-30.5-kDa bands of the OMPs isolated after Sarkosyl extraction from *H. ducreyi* strains 4, 6, 8, 47, 35000 and M, were most heavily radiolabelled by the Iodagen method. None of the radiolabelled surface-exposed polypeptides corresponded to any of the *H. ducreyi*-specific polypeptides identified by our MAbs. Since the labelling procedure would identify only those surface components that have accessible tyrosine and tryptophan residues for solid-phase iodination, outer-membrane preparations were also prepared by the Sarkosyl extraction method^{13,14} and separated on SDS-PAGE to identify all OMPs, including those that may not be surface exposed. The SDS-PAGE profile of the Sarkosyl-insoluble material demonstrated four major protein bands (fig. 3A, lane 6), which corresponded to four of the major bands identified by surface labelling (fig. 3A, lane 9). None of these four major OMPs had apparent mol. wts corresponding to those recognised by the *H. ducreyi*-specific MAbs (fig. 1). Therefore, based on the working definition of solid-phase iodination as the criterion for cell-surface exposure and Sarkosyl insolubility of the outer membrane, polypeptides B, C and D must not be presented on the cell surface or form part of the outer-membrane structure of *H. ducreyi*. It is interesting to note that while all three polypeptides exist in the soluble fraction of the cell, either in the cytosol or the periplasmic space, or both, a fraction of polypeptide D may be associated with the plasma membrane.

The fact that polypeptides A, B and D cross-reacted with MAb MHd078, suggested that they must carry the same epitope and are therefore likely to have

Fig. 4. A model of the sequence relationships of *H. ducreyi*-specific polypeptides as defined by the reactivities of the MAbs.

overlapping amino-acid sequences which are not shared by polypeptide C. Similarly, polypeptides B and C must have overlapping sequences (MHd082, MHd091) which are not shared by polypeptides A or D. The existence of an extensive but specific immune cross-reactivity suggested that these four polypeptides are members of a family of polypeptides which have overlapping sequences and that they may have been derived from the same precursor polypeptide or transcriptional unit. Based on the results reported here, we propose a sequence relationship between these four polypeptides, as shown in fig. 4. In this model, polypeptides A and C are derived from the opposite termini of a precursor without any overlap. Polypeptide D is derived from the centre and overlaps partially with polypeptide A on one side and polypeptide C on the other side and its internal portion does not overlap with polypeptides A or C. The sequence of part of polypeptide C must not overlap with other polypeptides, as indicated by the presence of a unique epitope defined by MHd086. Polypeptide B is placed with its left terminus between the left termini of polypeptides A and D, and its right terminus between the right termini of polypeptides B and D. This placement satisfies the existence of MAbs that recognise polypeptides A, B and D (MHd078), B and D (MHd080), B, C and D (MHd072, MHd107), B and C (MHd082, MHd091), and C alone (MHd086). To account for the existence of unique epitope(s) for polypeptide D (MHd083, MHd084), which has sequence homology with other polypeptides over its entire length, we propose that each of the two MAbs may depend on the α -amino or the carboxyl groups at either termini for recognition. Thus, all the observed epitope groups are accounted for without any inconsistencies. Based on this model, there are two other possible epitope groups, namely, a unique A epitope and a cross-reactive epitope A/B that are not represented in our panel of MAbs. The current model is

based on the premise that sequences in prokaryotic precursor polypeptides are contiguous since splicing mechanisms have not been reported. It may also be possible that these polypeptides are encoded by different genes with various degrees of homology, or have been derived from a large transcript with extensive repetitive sequences that are genetically non-contiguous or non-overlapping. Glycosylation and other post-translational modifications may also affect the reactivities with MAbs. It is possible that the variability in relative amounts of polypeptide A is due to it being a pre-protein or having variable degrees of post-translational modification. The question can be further studied when antisera directed against individual purified polypeptides become available.

The ubiquitous expression of these polypeptides and their highly conserved nature suggest that they may play an essential role in the biology of *H. ducreyi* which differs from other species of *Haemophilus*. Although the *H. ducreyi*-specific components A, B, C and D are not OMPs, this does not preclude them from having an active role in host-parasite interactions. The soluble nature of polypeptides C and D may allow some of these to have a role as extracellular factors that are either secreted or released through lysis of the bacterium. Such soluble virulence factors have been described in several bacterial pathogens. Indeed, the current vaccines for diphtheria and tetanus are based on soluble immunogens (toxins) secreted by these pathogens. Recent studies also indicate that ribosomes from *H. influenzae* are immunogenic and protective.^{16,17} Thus, the location on the OM is not an absolute requirement for the induction of immunity to the pathogen. Further studies are in progress to determine the function(s) of these highly conserved *H. ducreyi*-specific components. The specificity of these MAbs to *H. ducreyi* should also allow the development of sensitive immuno-diagnostics for chancroid. This has recently been recognised as an important sexually

transmitted disease that predisposes to infection by the human immunodeficiency virus (HIV) that causes the acquired immunodeficiency syndrome (AIDS).^{18,19}

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