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Antigens recognized by the human immune response to severe leptospirosis in Barbados

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SUMMARY

Serum samples obtained from patients hospitalized in Barbados with severe leptospirosis were tested by the microscopic agglutination test (MAT), enzyme immunoassay (EIA) and immunoblotting with leptospires that had been isolated from these patients. While serum samples taken a few days after onset of symptoms often showed no apparent correlation between MAT and EIA, later sequential serum samples produced similar profiles in both tests during the course of infection. Immunoblotting sonicate from *Leptospira interrogans* serovars *arborea*, *copenhageni* and *bim* with patients' sera, revealed reactions with a number of bands that corresponded with outer envelope components. These components included lipopolysaccharide (LPS), flagella and other outer membrane proteins, in addition to a low-molecular-weight (MW) carbohydrate cross-reactive with members of the Leptospiraceae. IgM antibodies elicited in the first to second week after infection reacted mainly with LPS and the low-MW cross-reactive carbohydrate. Comparative analysis of isolates of the same serovar by sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting showed that while two serovar *arborea* isolates were identical, serovar *bim* isolates differed significantly from each other. This difference was also observed in comparative MAT testing.

INTRODUCTION

Leptospirosis is an acute febrile illness, which varies in severity from mild to rapidly fatal. In contrast with the temperate regions of Australia and New Zealand where the most common form of leptospirosis is a mild self-limiting disease caused predominantly by *Leptospira interrogans* serovars *hardjo* and *pomona* [1, 2], leptospirosis on Barbados can be much more severe. A survey conducted on Barbados between 1979 and 1982 among patients admitted to hospital with severe leptospirosis revealed an average case rate of 17·6/100000 population per year and an overall case fatality rate of 18·8% [3]. Serological tests on patients' sera had highest titres to strains of the following serogroups:

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Autumnalis (72%), Icterohaemorrhagiae (20%), Ballum (6%), Canicola (1%) and Grippotyphosa (1%) [3]. Furthermore, isolates obtained from hospital patients on Barbados indicate that approximately 56% were serovar *bim*, 28% serovar *copenhageni*, 15% serovar *arborea* and 1% serovar *canicola* (Everard, unpublished results).

Leptospirosis is endemic on Barbados. Serological surveys have shown that 12·5% of school children had leptospiral antibodies at titres $\geqslant 50$ and an overall seropositive rate of 18·5% was found in a survey undertaken among rural and urban communities [4]. The incidence of severe or fatal leptospiral infection increases with age up to 60 years. The abundance of rats and domestic animals, the alkalinity of the Barbadian coralline soil and the warm humid tropical climate increase the likelihood of a continuing cycle of infection and reinfection [3].

Currently, there is no completely effective, non-toxic, leptospiral vaccine available internationally. Early whole-cell vaccines, while protective, often resulted in both local and severe systemic reactions due to growth in media containing animal serum [5]. While subsequent whole-cell vaccines produced in chemically-defined protein-free medium [6, 7] have reduced these reactions, they are much more difficult to produce. However, in the People's Republic of China human vaccination is undertaken at a national level using local serovars to produce efficacious vaccines in protein-free medium (Chen Ting-Zuo, personal communication).

Immunity to leptospirosis appears to depend solely on B-cell mediated antibody production [8], but the antigens involved in natural immunity to leptospiral infection are not well understood. We have previously reported [9] that LPS is a major antigen involved in the human immune response to infection with serovar *hardjo*. However, nothing is known about the major immunogens of leptospires found in tropical areas such as Barbados that may cause severe disease.

Thus in the present paper we sought to investigate, by immunoblotting, the antigens involved in the human antibody response to severe infections with serovars *arborea*, *copenhageni* and *bim*.

METHODS

Leptospires and serum samples

The leptospiral strains used were isolated from patients admitted to Queen Elizabeth Hospital, Bridgetown with suspected leptospirosis. All patients had pyrexia ($> 37{\cdot}7$ °C), nausea, anorexia and general aches and pains. In addition, 95% of patients also had jaundice [10]. *L. interrogans* serovars *arborea* (L264 and L268) and *bim* (L266 and L267) were isolated from blood samples, while serovar *copenhageni* (L265) was isolated from urine. Isolation and identification of leptospires was performed at the MRC/Government of Barbados Leptospira Laboratory, the Centers for Disease Control (CDC), Atlanta, USA, and The Royal Tropical Institute, Amsterdam, The Netherlands, using standard procedures and those previously described [3]. Other strains of leptospires used were obtained as previously described [11]. Leptospires were subsequently grown at 30 °C in Tween-albumin EMJH medium with added pyruvate [12] and washed three times

with phosphate-buffered saline, pH 7·2, to remove adherent bovine serum albumin. Blood samples were also taken during a period of approximately 3 weeks after admission to hospital and in some cases further samples were taken 12 months later.

Serological tests and monoclonal antibodies

The microscopic agglutination test (MAT) and enzyme immunoassay (EIA) were performed as previously described [7, 9]. Monoclonal antibodies MUM/F9-10/*copenhageni* and MUM/F1-1/*copenhageni* were produced from BALB/c mice immunized with serovar *copenhageni* as previously described [11, 13].

Preparation of leptospiral antigens

Ultrasonicated leptospiral antigens and outer envelope preparations were prepared and standardized as previously described [9, 11, 14]. Where required, leptospiral antigen samples were digested with proteinase K [15] before polyacrylamide gel electrophoresis (SDS–PAGE) or blotted antigens were treated before immunostaining with proteinase K, 100 μg/ml in PBS for 24 h at 37 °C.

Gel electrophoresis and immunoblotting

Samples standardized for protein content were subjected to SDS–PAGE and then either stained with 0·25% Coomassie blue [9], silver stained for LPS [15], or transblotted onto nitrocellulose (Schleicher and Schüll, pore size 0·45 μm) at 60 V for 3 h using the buffer of Towbin [16] diluted 1:2. Human sera or mouse monoclonal antibodies and the appropriate peroxidase-conjugated anti-immunoglobulins were consecutively incubated with the blots which were then developed with 4-chloro-1-naphthol [9].

RESULTS

SDS–PAGE profiles of Barbadian leptospiral isolates

Thirty μg of ultrasonicated leptospires (sonicates) were separated by SDS–PAGE and stained with either Coomassie blue or LPS silver stain (Fig. 1). Staining with Coomassie blue revealed a complex pattern of protein bands with molecular weights (MW) ranging from 14·4 to > 94 kilodaltons (kDa). Two isolates of serovar *arborea* (L264 and L268) were compared and found to have identical protein profiles, while comparison of two isolates of serovar *bim* (L266 and L267) showed significant differences in their protein profiles in the 40 kDa region. Only one isolate of serovar *copenhageni* (L265) was obtained from Barbados, and this was compared with a laboratory strain of serovar *copenhageni* (L136). These two isolates shared a number of proteins, but some quantitative differences were apparent. Overall, a number of proteins were shared by all isolates of the three different serovars tested (Fig. 1).

Determination of SDS–PAGE LPS profiles by LPS silver staining (Fig. 1) showed that serovar *copenhageni* produced two diffuse bands of 14·4–20 and 26–35 kDa. The two isolates of serovar *arborea* produced an identical LPS profile with three major bands of 16, 23 and 29 kDa. However, the two isolates of serovar *bim* appeared to differ greatly, with isolate L266 producing a smear from

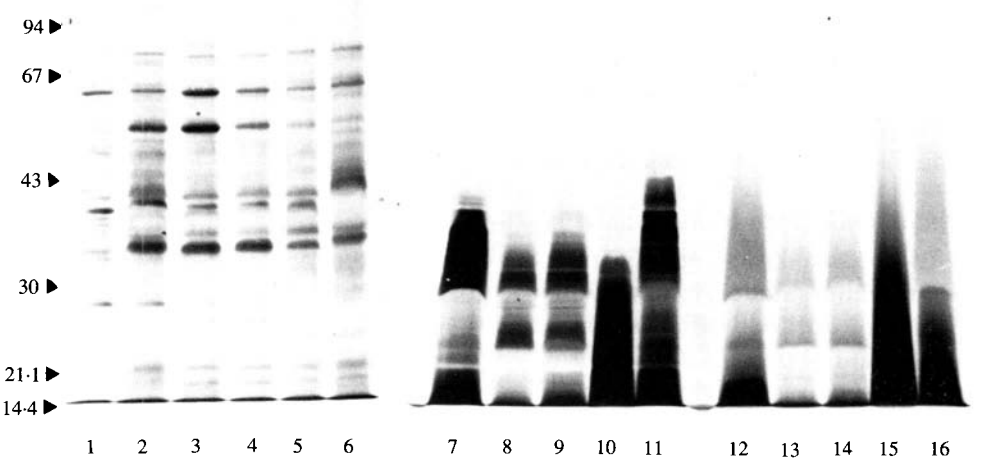

Fig. 1. SDS–PAGE profiles of sonicated Barbadian leptospiral isolates stained with either Coomassie blue (lanes 1–6) or LPS silver stain (untreated, lanes 7–11; proteinase K-treated, lanes 12–16). Lane 1, *L. interrogans* serovar *copenhageni* (L136); lanes 2, 7 and 12 *L. interrogans* serovar *copenhageni* (L265); lanes 3, 8 and 13, *L. interrogans* serovar *arborea* (L264); lanes 4, 9 and 14. *L. interrogans* serovar *arborea* (L268); lanes 5, 10 and 15, *L. interrogans* serovar *bim* (L266); lanes 6, 11 and 16, *L. interrogans* serovar *bim* (L267). Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

14·4–30 kDa, while isolate L267 appeared to have two major diffuse components at 14·4–20 and 27–34 kDa. Similar profiles were also observed when the sonicates were digested with proteinase K prior to SDS–PAGE and LPS silver staining (Fig. 1), although the intensity was slightly reduced, particularly with serovar *bim* L267.

Analysis of antibodies at different stages of infection

Serial sets of sera, from three patients, were obtained where the identity of the infecting leptospiral serovar was proved by culture. These sera were tested by MAT, EIA and immunoblotting against the leptospiral isolates obtained from the corresponding patients. Table 1 shows the time-course of antibody appearance in patients 1–3 when tested by MAT and EIA against the homologous infecting leptospiral isolate. Sera from Patient 1 (Table 1) tested against serovar *arborea* (L264) showed that both IgM and IgG antibody levels increased significantly between 6 and 8 days after onset of symptoms. However, during this period there was no corresponding MAT titre against the infecting serovar. The MAT titre, as well as IgM and IgG antibody levels all increased between days 8 and 27. IgM and IgG levels as well as the MAT titre had all declined significantly by the 12-month follow-up sample.

Sera from patient 2 (Table 1) when tested against the infecting isolate of serovar

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Table 1. *Time-course of appearance of antibodies measured by MAT and ELA*

Patient	Day after onset of symptoms	MAT titre	EIA OD(488 nm)* IgM	IgG
1	6	< 20	0·16	0·56
	8	< 20	0·56	1·78
	27	1280	2·00	2·00
	365	80	0·14	0·28
2	5	80	> 2·00	0·11
	10	1280	> 2·00	0·17
	25	5120	> 2·00	0·46
3	6	80	0·28	0·00
	9	10240	1·24	0·70
	25	2560	1·24	0·99
	365	1280	0·40	1·06

* Adjusted for background.

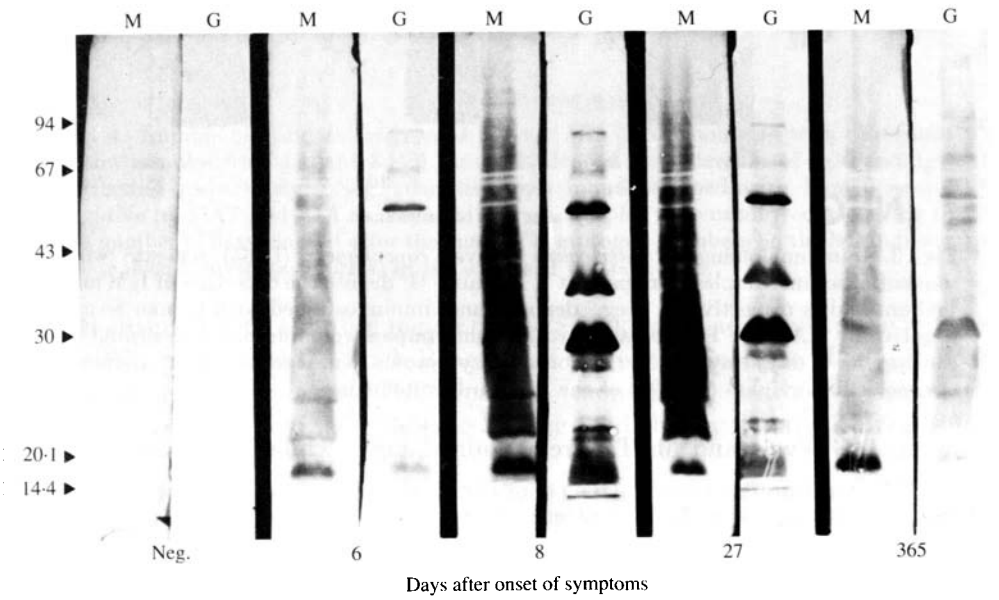

Fig. 2. Immunoblotting *L. interrogans* serovar *arborea* (L264) sonicate with sequential serum samples from patient 1. 'M' and 'G' denote the detection of IgM and IgG antibodies respectively. 'Neg.' denotes lanes immunostained with human serum negative by MAT and EIA as a control. Serum samples were numbered according to the number of days elapsed after the onset of symptoms. Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

copenhangeni (L265) showed IgM was at a maximum 5 days after onset of symptoms, while IgG levels were significantly less. MAT titre and levels of IgM (not shown) and IgG all increased between days 5 and 25. Sera from patient 3 (Table 1) when tested against the infecting isolate of serovar *bim* (L267) showed that both MAT titre and IgM levels were elevated by day 9. The MAT titre declined slightly between days 9 and 25, while IgM levels remained constant. In contrast, IgG levels steadily increased between days 6–25. At the 12-month

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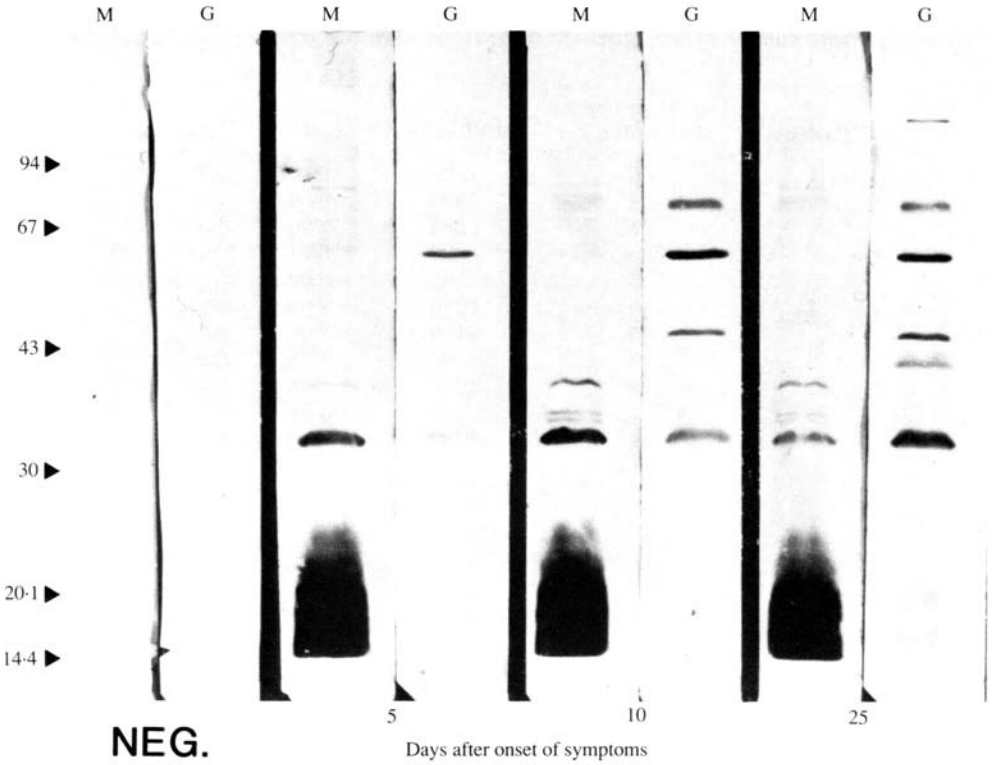

Fig. 3. Immunoblotting *L. interrogans* serovar *copenhageni* (L265) sonicate with sequential serum samples from patient 2. 'M' and 'G' denote the detection of IgM and IgG antibodies respectively. 'Neg.' denotes lanes immunostained with human serum negative by MAT and EIA, as a control. Serum samples were numbered according to the number of days elapsed after the onset of symptoms. Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

follow-up IgG levels and MAT titre remained high, while IgM levels declined significantly.

Immunoblotting sera from patient 1 with the corresponding homologous *arborea* sonicate (Fig. 2) showed that from day 6 IgM antibodies reacted with a diffuse 14·4 > 94 kDa smear, although the 20, 23 and 27 kDa components shown by LPS silver staining could be observed. However, IgG antibodies did not produce a similar pattern until the day 365 sample. Instead they reacted with a number of discrete bands of 20, 22, 23, 28, 32, 38, 55 and 90 kDa. Similar results were obtained immunoblotting patient 4 sera (Fig. 5, lanes 1–2) with its corresponding homologous *arborea* (L268) isolate.

Immunoblotting sera from patient 2 (Fig. 3) with its corresponding homologous *copenhageni* sonicate showed that by day 10 IgM antibodies reacted strongly with a diffuse band of 14·4–24 kDa and with other bands of 32, 38, 59 and 72 kDa, as well as a 34·5–35 kDa doublet. In contrast, IgG antibodies reacted only slightly with the diffuse 14·4–24 kDa band but strongly with bands of 32, 38, 44, 59 and 72 kDa. By day 25, IgM also reacted slightly with a diffuse band of approximately 26–35 kDa. A similar reaction was observed with serum from patient 5 (Fig. 5, lanes 4–5) that was also MAT positive to serovar *copenhangeni*. IgM antibodies

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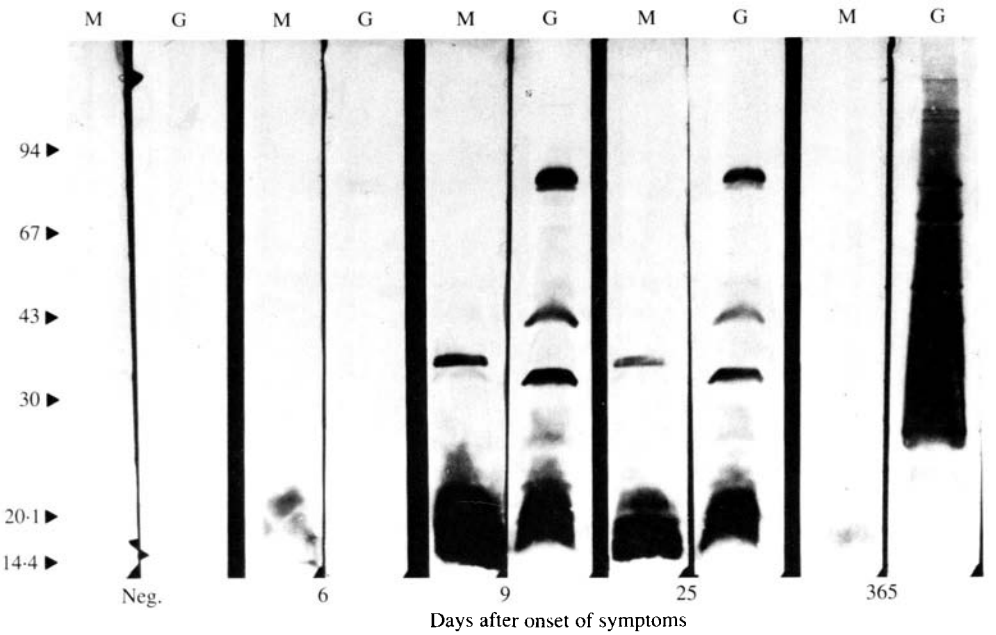

Fig. 4. Immunoblotting *L. interrogans* serovar *bim* (L267) sonicate with sequential serum samples from patient 3. 'M' and 'G' denote the detection of IgM and IgG antibodies respectively. 'Neg.' denotes lanes immunostained with human serum negative by MAT and EIA as a control. Serum samples were numbered according to the number of days elapsed after the onset of symptoms. Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

reacted strongly with a diffuse region of 26–> 94 kDa, in addition to the reaction with a diffuse 14·4–24 kDa band. Reaction of serovar *copenhageni* (L265) sonicate with a serovar *copenhageni* LPS-reactive monoclonal antibody, designated MUM/F1-1/*copenhageni*, (Fig. 5, lane 3) suggested that reaction with the diffuse 26–94 kDa region was due to LPS antibodies.

Immunoblotting sera from patient 3 (Fig. 4) with the corresponding homologous *bim* sonicate (L267) showed that from day 9 IgM antibodies reacted with a diffuse 14·4–22 kDa region, a 32 kDa band and a tight 34·5–35 kDa doublet. IgG antibodies also reacted with the diffuse 14·4–22 kDa region, as well as bands of 32, 38 and 75 kDa. In contrast, the day 365 sample showed very little IgM reactivity, but strong IgG reactivity with a diffuse region of 25–> 94 kDa; the lower portion of this region was similar in size and shape to the region stained by LPS silver staining (Fig. 1). Immunoblotting sera from patient 6 (which reacted with serovar *bim* by MAT), with L267 sonicate showed that IgM antibodies reacted with two diffuse regions of 14·4–25 and 25–4 kDa, while IgG antibodies reacted with the diffuse 14·4–25 kDa region in addition to bands of 28, 45 and 85 kDa as well as a 34–35 kDa doublet (Fig. 5, lanes 6, 7).

Identification of some antigens

A monoclonal antibody, designated MUM/F9-10, that reacted with a major outer envelope protein of all serovars of *L. interrogans* tested [11], was immunoblotted with sonicates from Barbadian isolates of serovars *arborea*,

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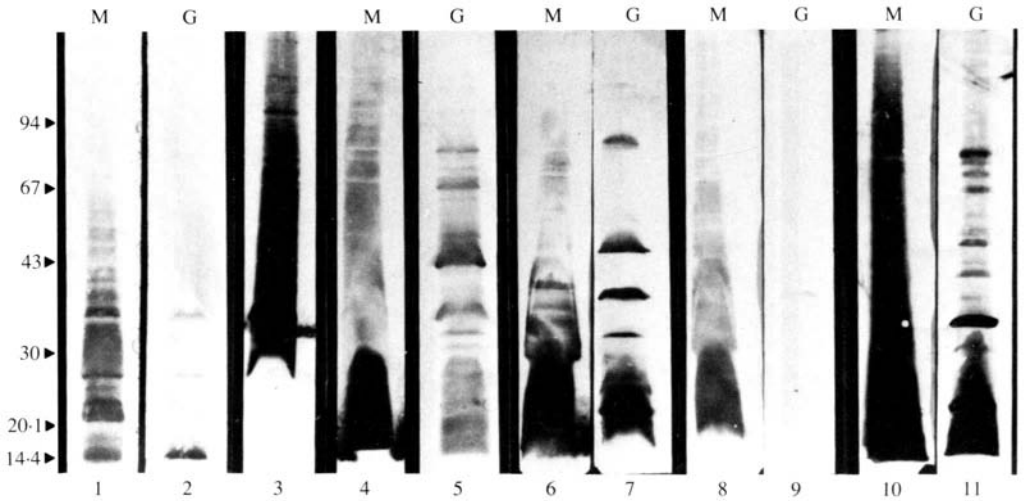

Fig. 5. Immunoblotting *L. interrogans* serovar *arborea* (L268) sonicate (lanes 1 and 2), *L. interrogans* serovar *copenhageni* (L265) sonicate (lanes 3–5), *L. interrogans* serovar *bim* sonicate (isolate L267, lanes 6–9 and isolate L266, lanes 10 and 11) with monoclonal antibodies and human sera. 'M' and 'G' denote the detection of IgM and IgG antibodies respectively. Lanes 1 and 2, patient 4 serum (day 24); lane 3 monoclonal antibody MUM/F1-1; lanes 4 and 5, patient 5 serum (day 21); lanes 6 and 7, patient 6 serum (day 34); lanes 8–11, patient 7 serum (day 8). Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

copenhageni and *bim* (Fig. 6, lanes 1–3) and reacted in all cases with a major band of 32 kDa. Immunoblotting a serovar *copenhageni* outer envelope preparation (Fig. 6, lanes 4–9) with sera from Barbadian patients that were MAT positive to serovar *copenhageni*, showed that a number of immunogenic components were present in the outer envelope including LPS and the 32 kDa major outer envelope protein, as well as the diffuse 14·4–25 kDa band and bands of 29, 34·5, 35, 38, 59, 82 and 83 kDa. Similar reactivities with the protein antigens of serovar *copenhageni* outer envelope were also observed with sera from patients 1, 3, 4, 5 and 6 (results not shown).

Immunoblotting serovar *arborea* sonicate with monoclonal MUM/F9-10 before (Fig. 6, lane 10) and after (Fig. 6, lane 11) treatment with proteinase K showed that reactivity with the 32 kDa outer envelope protein was completely lost upon treatment with proteinase K. Similarly, treatment of blotted serovar *arborea* sonicate with proteinase K before reacting with day 27 sera from patient 1, showed that the IgM profile was unchanged (Fig. 6, lane 12) while IgG reactivity with all discrete bands previously seen in Fig. 2 was lost (Fig. 6, lane 13).

Cross-reactivity between serovars

Sera from patients whose IgM antibodies reacted with a diffuse, low-MW band of 14·4 to approximately 25 kDa were immunoblotted with homologous and heterologous leptospiral sonicates that had been treated with proteinase K prior to SDS–PAGE. Fig. 7 shows that sera from patients 2 and 3 reacted with diffuse low-MW material not only from homologous and heterologous *L. interrogans*

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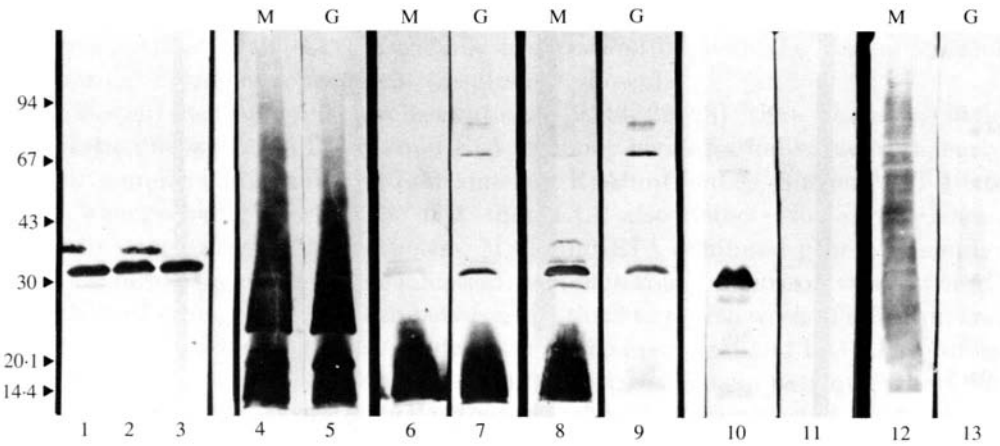

Fig. 6. Immunoblots of *L. interrogans* serovar *arborea* (L264) sonicate (untreated, lanes 1 and 10; proteinase K-treated, lanes 11–13), *L. interrogans* serovar *copenhageni* (L265) sonicate (lane 2), *L. interrogans* serovar *bim* (L267) sonicate (lane 3) and *L. interrogans* serovar *copenhageni* (L45H) outer envelope preparation (lanes 4–9) with monoclonal antibodies and human sera. 'M' and 'G' denote the detection of IgM and IgG antibodies respectively. Lanes 1–3 monoclonal antibody MUM/F9-10 (anti-membrane protein); lanes 4 and 5, patient 8 serum; lanes 6 and 7, patient 9 serum; lanes 8 and 9, patient 2 serum; lanes 10 and 11, monoclonal MUM/F1-1 (anti-LPS); lanes 12 and 13, patient 1 serum. Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

serovars, but also with *L. biflexa* serovar *patoc* and *Leptonema illini*. Serum from patient 6, that was MAT positive to serovars *arborea* and *bim*, but not serovar *copenhageni*, reacted with the diffuse 14·4–25 kDa band from all serovars. However, it reacted with the LPS of serovars *arborea* and *bim* only (Fig. 7, panel 4).

Differences between serovar bim *isolates*

Two isolates of serovar *bim* from Barbados were available for this study. Isolates L266 and L267 were obtained from patients 7 and 3 respectively. Both isolates were confirmed as serovar *bim* by the CDC, Leptospira Reference Laboratory, Atlanta, USA and by monoclonal antibody analysis (H. Korver, personal communication).

As shown previously (Fig. 1), L266 and L267 differed markedly in their LPS silver staining profiles. Consequently, sera from patients 3 and 7 were immunoblotted with both L266 and L267 to determine if these differences reflected antigenic differences. Immunoblotting sera from patient 7 (Fig 5, lanes 8–11) showed that the different IgM reactivity patterns between the two serovar *bim* isolates were similar to their respective LPS silver stain profiles. Similar results were obtained with patient 3 sera (results not shown). Furthermore, testing sera from a number of Barbadian patients, who were MAT positive to serovar *bim*, with both L266 and L267 revealed that while some sera reacted equally with both isolates, others gave higher MAT titres against isolate L266 (Table 2, not all sera shown).

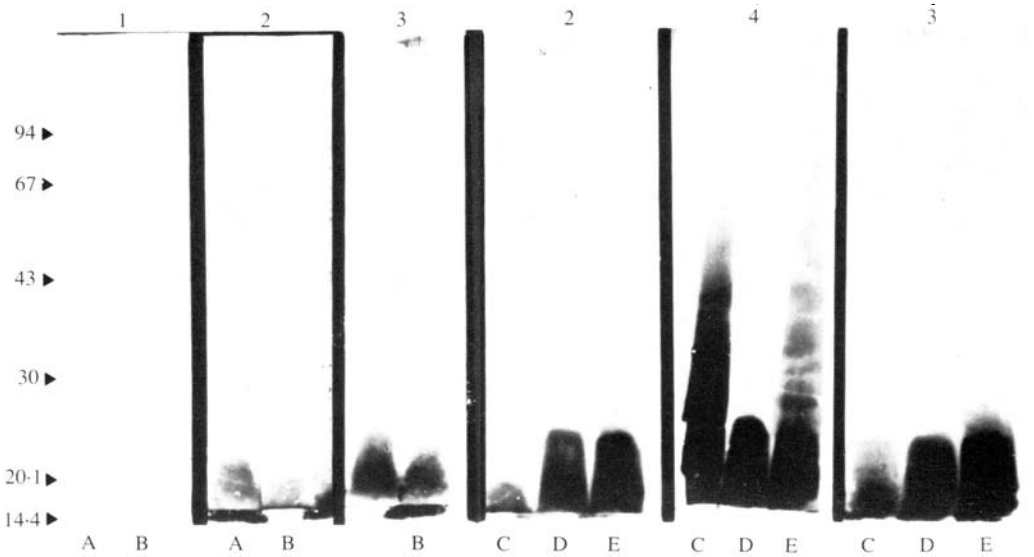

Fig. 7. Immunoblots of proteinase K-treated leptospiral sonicates with negative control serum (1); patient 2, day 5 serum (2); patient 3, day 9 serum (3) and patient 6, day 8 serum (4). Lane A, *Leptonema illini*; lane B, *L. biflexa* serovar *patoc*; lane C, *L. interrogans* serovar *arborea* (L264); lane D, *L. interrogans* serovar *copenhageni* (L265) and lane E, *L. interrogans* serovar *bim* (L267). Only IgM antibodies were detected. Numbers on the left indicate the molecular weights (in kDa) of the standard proteins used.

Table 2. *Comparing serum MAT titres derived from testing with two isolates of* L. interrogans *serovar* bim

Serum	Day after onset of symptoms	MAT titre L266	MAT titre L267
Patient 9	6	320	320
	9	40960	5120
	26	40960	2560
	364	640	1280
Patient 3	6	80	80
	9	10240	10240
	25	5120	2560
	365	320	1280

DISCUSSION

The identity of the infecting serovar can only be proved by culture as the highest MAT titre does not always reflect the infecting serovar [12], and human sera cross-react widely by MAT especially during the acute stage of illness [18, 19]. In these studies autologous or local isolates were used in order to minimize variation in the antigens identified by patients' sera [9, 20, 21]. In SDS–PAGE analysis of the five serum-matched leptospiral isolates used in this study the protein profiles of two *arborea* isolates were identical, but the two *bim* isolates showed some differences, and there were only minor differences between the *copenhageni* isolate and a laboratory strain.

However, the LPS profiles of the two *arborea* isolates were identical, but there were differences in the LPS of the *bim* isolates and between the Barbadian and laboratory strains of *copenhageni* (results not shown).

Consistent with previous observations [9, 14, 22, 23] that there is little correlation between MAT titres and EIA antibody levels for individual sera, early serum samples had significant IgM and IgG EIA antibodies, but no MAT titre. This was probably due to the fact that EIA also detects sub-surface, non-agglutinating antigens. Nevertheless, MAT and EIA exhibited generally similar profiles during the course of the infection, although this comparison was restricted by a lack of serum samples taken between the third to fourth week of infection and the 12-month follow-up sample. Differences in the early IgM and IgG EIA profiles (Table 1) may indicate a previous or secondary exposure as in patient 1 where the level of IgG was higher than IgM. However, this was not reflected by the MAT titre on days 6 and 8. EIA analysis of the 12-month follow-up samples suggested a possible reinfection in patient 3, as while IgM levels decreased markedly between days 25 and 365, the MAT titre and IgG levels decreased only slightly or increased respectively, in contrast to patient 1 whose MAT titre, IgM and IgG levels had all decreased significantly. However, subsequent long-term follow-up studies have shown that MAT titres $\geqslant 400$ may persist for a year or longer and reinfection is not uncommon [24].

Immunoblotting serovar *arborea*, *copenhageni* and *bim* sonicates with sera from patients 1–3 respectively, showed little obvious change in the antigens detected after the second to fourth week of infection, although a lack of serum samples during this period makes this conclusion uncertain. However, we have previously shown that there was little change in the antigens detected after the second to third week of infection with serovar *hardjo* [9].

Immunoblotting serovar *arborea* sonicate with sera from patients 1 and 4, showed that IgM reacted with a diffuse smear, presumed to be LPS, because of its similarity to silver stained LPS and its stability to proteinase K. IgG reactivity appeared to be confined to a number of protein bands until the day 365 sample where weak reaction with LPS was observed.

Immunoblotting serovar *copenhageni* sonicate with sera from patients 2 and 5 showed variable reactions with LPS, the identity of which was proved with a serovar *copenhageni* LPS-reactive monoclonal antibody, and also by the location, shape and proteinase-K resistance of the LPS. IgM reacted strongly with a diffuse 14·4–24 kDa band, and also with the 32 kDa major outer envelope protein, in addition to a 34·5–35 kDa doublet, previously shown to be of flagellar origin [9, 24]. IgG antibodies mainly reacted with a number of discrete bands ranging in MW from 32–72 kDa. IgM from patient 3 reacted only with the diffuse 14·4–22 kDa band, the 32 kDa major outer envelope protein and the 34·5–35 kDa flagellar doublet. IgM from patient 6, in addition to similar reactions, also reacted strongly with a diffuse 35–41 kDa band corresponding to the LPS profile shown by LPS silver staining. The contention above that patient 3 had a reinfection was supported by the strong IgG reactivity against serovar *bim* LPS, which we have shown to be a major immunogen in current infections caused by other leptospiral serovars [7, 9, 11].

Immunoblotting the two isolates of serovar *bim* (L266 and L267) with specific

antisera obtained from the same patients showed that while the two isolates had different LPS profiles, antibodies elicited against each isolate could react with LPS from both isolates. However, some sera had higher MAT titres against *bim* isolate L266 than L267. This appeared to indicate that L266 possessed additional agglutinating MAT epitope(s) not found in L267, an observation which may be important for protection and therefore for vaccine production.

The low-MW proteinase-K resistant material which reacted with patient 2, 3 and 6 sera was apparently genus reactive and not protein. We have previously reported similar cross-reactive material [26] that was periodate-, but not proteinase K-sensitive. As genus-reactive tests have been found to be positive earlier than the MAT [17] and as all patients produced IgM antibodies to this low MW material, as early as the first week after onset of symptoms, this material may be useful in the early diagnosis of leptospirosis through a genus-reactive test.

Our results show that LPS and other outer envelope components, such as flagella and the 32 kDa major outer envelope protein, are significant major antigens involved in the human antibody response to infection with *L. interrogans* serovars *arborea*, *copenhageni* and *bim* in Barbados. Most of the outer envelope proteins and the low MW carbohydrate antigen were shown to be cross-reactive antigens, of which the low MW carbohydrate antigen may be useful in an early genus-reactive diagnostic test. As LPS elicits opsonic and protective antibodies [13, 27–29, 30], lacks endotoxic activity [31] and has been shown to be a major protective antigen involved in the human antibody response to infection with other leptospiral serovars [7, 9], LPS components may be useful as immunizing agents. Studies are currently being undertaken to further define the components of LPS and other antigens involved in the immune response to infection with leptospires.

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