Comparison of outer-membrane proteins of *Pasteurella haemolytica* expressed *in vitro* and *in vivo* in cattle

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Outer-membrane protein (OMP) profiles of two serotype A1 isolates of *Pasteurella haemolytica* were compared by SDS-PAGE and Western blotting with bovine convalescent serum after growth (a) *in vitro* under iron-sufficient and -deficient conditions, (b) *in vivo* in the lungs of experimentally infected calves and (c) *in vivo* in diffusion chambers implanted into the peritoneal cavities of calves. Lung-grown bacteria differed from iron-sufficient *in vitro*-grown bacteria in having enhanced expression of the previously recognized 71, 77 and 100 kDa iron-regulated proteins, reduced expression of 18, 31, 39.5 and 50 kDa proteins, and expression of a 19 kDa protein. Differences were also apparent in the Western blot profiles of OMPs of *in vitro*- and lung-grown bacteria. These included the apparent lack of recognition of the 100 kDa protein in the lung-grown bacteria, but not in the *in vitro*-grown bacteria, and more intense staining of a 47 kDa protein in *in vitro*-grown bacteria, but not in lung-grown bacteria. The OMP profiles of the chamber-grown bacteria resembled those of the lung-grown bacteria in that expression of the 18, 19, 31 and 39.5 kDa proteins was similar. These similarities demonstrated that the chamber-grown bacteria had adapted to the *in vivo* environment, and that growth conditions within the chambers resembled, but not perfectly, those within the lungs. For example, expression of the three iron-regulated OMPs was very low in the chamber-grown bacteria compared to the lung-grown bacteria. The OMP profiles of bacteria grown *in vitro* in newborn calf serum closely resembled those of lung-grown bacteria, suggesting that *in vivo* growth may be partly reproduced *in vitro* by growing the bacteria in newborn calf serum.

Keywords: *Pasteurella haemolytica*, outer-membrane proteins, bovine pneumonic pasteurellosis

INTRODUCTION

Pasteurella haemolytica is an economically important respiratory pathogen of cattle, responsible for pneumonic pasteurellosis, a severe fibrinous pleuropneumonia (Frank, 1989). The bacterium also causes pneumonia and septicaemia in sheep (Gilmour & Gilmour, 1989). Bovine pneumonic pasteurellosis is due predominantly to isolates of serotype A1, whereas the single most predominant serotype from pneumonic sheep is A2 (Frank, 1989; Gilmour & Gilmour, 1989). Vaccines against bovine

pneumonic pasteurellosis, including live, killed and extract vaccines, have not been particularly effective in reducing losses (reviewed by Mosier *et al.*, 1989). Very little is known about the role of the different cell-surface components, including the capsule, lipopolysaccharide (LPS) and outer-membrane proteins (OMPs), in the pathogenesis of pneumonic pasteurellosis. Recent research has attempted to identify important immunogenic components of *P. haemolytica*, such as LPS (Confer *et al.*, 1986; Davies *et al.*, 1991; Ali *et al.*, 1992) and various OMPs (Nelson & Frank, 1989; Knights *et al.*, 1990; Craven *et al.*, 1991; Davies *et al.*, 1992; McCluskey *et al.*, 1994), including iron-regulated OMPs (Donachie & Gilmour, 1988; Deneer & Potter, 1989; Gilmour *et al.*,

Abbreviation: OMP, outer-membrane protein.

1991). However, these investigations have involved the analysis of *in vitro*-grown organisms as opposed to *in vivo*-grown organisms.

It is now well recognized that pathogenic bacteria may express different cell-surface components when growing *in vivo* and *in vitro* (Brown & Williams, 1985; Smith, 1990). For example, pathogenic Gram-negative bacteria may produce a number of iron-regulated OMPs when growing *in vivo* which are not produced, or are produced less abundantly, when growing *in vitro* under iron-sufficient conditions (Griffiths *et al.*, 1983; Sciortino & Finkelstein, 1983; Brown *et al.*, 1984). *P. haemolytica* produces three iron-regulated OMPs of 71, 77 and 100 kDa under iron-restricted *in vitro* growth conditions (Donachie & Gilmour, 1988; Deneer & Potter, 1989; Davies *et al.*, 1992). The 100 kDa protein has been demonstrated to be the receptor for bovine transferrin (Ogunnariwo & Schryvers, 1990). Previous studies on the *in vivo* expression of OMPs in *P. haemolytica* have produced conflicting results regarding the expression of the iron-regulated OMPs. These differences may be due to strain or serotype variations, to the use of different animals for the *in vivo* studies, or to the growth characteristics of the bacteria at different sites. Morck *et al.* (1991) demonstrated *in vivo* expression of the 71, 77 and 100 kDa OMPs in a serotype A1 isolate grown in a peritoneal implant chamber in a rabbit: all three proteins were recognized by bovine convalescent antiserum. In a study of a serotype A2 isolate, grown in the pleural fluid of a sheep, Donachie & Gilmour (1988) demonstrated *in vivo* expression of 71 and 100 kDa OMPs, but not of a 77 kDa OMP. These authors, furthermore, demonstrated antibody recognition of the 71 kDa OMP, but not of the 100 kDa OMP in lamb convalescent antiserum. Confer *et al.* (1992) examined whole-cell profiles of serotype A1 cells obtained from a subcutaneous chamber implanted into a calf, and were able to detect differences between *in vitro*- and *in vivo*-grown cells only in proteins of 24, 26 and > 150 kDa, but it was not confirmed whether these proteins were located in the outer membrane. Sutherland *et al.* (1990) examined the whole-cell profiles of a serotype A2 isolate, grown in implant chambers in the peritoneal cavity of a sheep, and demonstrated enhanced production of 71 and 100 kDa proteins; these proteins were not, however, recognized by antibodies in Western blots.

None of the above *in vivo* studies attempted to examine the expression of OMPs of *P. haemolytica* obtained directly, without subculture, from the infected lungs of calves, or to correlate the findings obtained from *in vivo* implant chambers with findings from infected lungs. Although an *in vivo* implant chamber may provide a more natural environment than a flask of broth, the conditions within a chamber may still not exactly mimic the conditions occurring at the site of the natural infection. Therefore, the objectives of the present study were twofold. Firstly, to compare the OMP profiles of two serotype A1 isolates of *P. haemolytica* grown in intraperitoneal implant chambers in healthy calves, with those of the same isolates obtained from the lungs of experimentally infected calves. Secondly, to compare the OMP profiles of *in vivo*-grown

bacteria with the profiles of the same bacteria grown under various *in vitro* conditions as described previously (Davies *et al.*, 1992), and thereby determine the *in vitro* growth conditions most able to reproduce the *in vivo* growth conditions.

METHODS

Bacterial isolates and *in vitro* growth conditions. Two serotype A1 isolates of *P. haemolytica* were used in the present study, PH2 (S/C 82/1) and PH10. Both isolates were obtained from confirmed cases of bovine pneumonic pasteurellosis, and possessed smooth LPS of type 1 (Davies *et al.*, 1991; Ali *et al.*, 1992).

Bacteria were stored at -70°C in brain heart infusion broth (BHIB; Oxoid) containing 50% (v/v) glycerol and routinely subcultured on brain heart infusion agar (BHIA; Oxoid) containing 5% (v/v) defibrinated sheep's blood. For chamber inoculations and outer-membrane preparations, bacteria were grown to early stationary phase (approximately 6 h) in BHIB. All incubations were carried out at 37°C and broth cultures were shaken at 120 r.p.m. For iron-deficient *in vitro* growth, bacteria were grown in BHIB containing $20\text{ }\mu\text{M}$ ethylenediaminedihydroxyphenylacetic acid (EDDA) (Davies *et al.*, 1992).

Calves. Four-month-old weaned, dairy-cross calves were used in the experiments. Prior to use in chamber and lung infection experiments, calves were screened by indirect haemagglutination assay for antibodies to serotypes A1 and A2 (Shreeve *et al.*, 1972).

In vivo culture of bacteria

(i) **Lungs.** Bacteria were grown in 200 ml BHIB to late logarithmic phase (4–5 h), harvested by centrifugation at $10000\times g$ for 20 min and resuspended in 20 ml PBS (150 mM NaCl, 7.2 mM Na_2HPO_4 , 2.8 mM NaH_2PO_4 , pH 7.2). Calves were infected by the intra tracheal route as described previously (Gibbs *et al.*, 1984), killed after 18 h and exsanguinated, the lungs removed, and bacteria washed from the airways in PBS. Bacteria were recovered from the lung washings by differential centrifugation. Large debris and blood cells were removed by three centrifugations at $500 g$ for 5 min, and bacteria harvested by centrifugation at $10000 g$ for 30 min and resuspended in 20 mM Tris/HCl (pH 7.2) for outer-membrane preparation. Viable counts were performed on the challenge suspension as described previously (Davies *et al.*, 1994b).

(ii) **Implant chambers.** Bacteria were obtained from intraperitoneal diffusion chambers implanted into calves as described previously (Davies *et al.*, 1994b). Due to the slow decline in bacterial numbers within the chambers over a 10 d period (Davies *et al.*, 1994b), the chambers were inoculated with a high number of bacteria to give an initial cell density of approximately 5×10^8 c.f.u. ml^{-1} . The bacteria within the chambers were recovered after 18 h. Using this method, sufficient numbers of bacteria were obtained to allow preparation of outer membranes.

Preparation of outer membranes. Outer membranes were obtained by Sarkosyl extraction as described previously (Davies *et al.*, 1992).

Protein assay. Protein concentrations of outer membrane samples were assayed by the modified Lowry procedure of Markwell *et al.* (1978).

SDS-PAGE. OMPs were separated by SDS-PAGE using the SDS-discontinuous system of Laemmli (1970) as described

Fig. 1. Coomassie-blue-stained SDS-PAGE OMP profiles (a) and Western blot (b) of *in vitro*- and lung-grown cells of *P. haemolytica* isolates PH2 (lanes 1-3) and PH10 (lanes 4 and 5). Bacteria were grown in BHIB (lanes 1 and 4), BHIB supplemented with 20 μ M EDDA (lane 2), and in calf lungs (lanes 3 and 5). Size markers are shown in lane 6 in (a).

previously (Davies *et al.*, 1992). Approximately 20 μ g OMP were loaded per well, and visualized by staining with Coomassie blue. Molecular mass standards (Pharmacia) were phosphorylase b (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α -lactalbumin (14.4 kDa).

Western blotting. Differences in immune reactivity to *in vitro*- and *in vivo*-expressed OMPs were investigated by Western blotting as described previously (Davies *et al.*, 1994a). The primary antibody source was convalescent antiserum raised by experimental infection of a calf (Ali *et al.*, 1992). The antiserum was used at a dilution of 1:200 in antibody buffer.

RESULTS

Sufficient numbers of cells of *P. haemolytica* isolates PH2 and PH10 were obtained from both infected lungs and implant chambers to extract outer membranes. OMP profiles, by SDS-PAGE, of cells obtained directly, without subculture, from the lungs of infected calves, and from implant chambers, were compared with each other and with OMP profiles of cells grown *in vitro* under iron-sufficient and -deficient conditions. OMP profiles of *in vitro*- and lung-grown cells of isolates PH2 and PH10 are shown in Fig. 1(a). Isolates PH2 and PH10 were serotype

A1 isolates which have previously been shown to have slight differences in virulence but similar OMP profiles (unpublished observations). During *in vitro* growth, these isolates showed variation in the expression of the 40 kDa OMP (Fig. 1a, lanes 1 and 4), although it should be noted that expression of this protein can vary within the same isolate according to the degree of aeration during growth (Davies *et al.*, 1992). Isolates PH2 and PH10 also exhibited some variation in their Western blot profiles, during both *in vitro* and *in vivo* growth (Fig. 1b, lanes 1 and 4, and 3 and 5). Important differences in the SDS-PAGE profiles between the *in vitro*- and lung-grown cells included the reduced expression of the 31 and 39.5 kDa major proteins of both isolates in the lung-grown cells (Fig. 1a, lanes 3 and 5). There was enhanced expression of the 71, 77 and 100 kDa iron-regulated proteins in the lung-grown cells of isolate PH10 (Fig. 1a, lanes 4 and 5), but this was not as evident in isolate PH2 (Fig. 1a, lanes 1 and 3). Expression of these proteins, especially the 71 kDa protein, was not as great as when the cells were grown in BHIB in the presence of EDDA (Fig. 1a, lane 2). Further differences between the *in vitro*- and lung-grown profiles included reduced expression of the 18 kDa protein, and the appearance of an additional protein of 19 kDa in the lung-

Table 1. Expression of OMPs from *in vitro*- and *in vivo*-grown *P. haemolytica*

Expression of OMPs was graded as follows: + + +, high; + +, moderate; +, low; -, none.

Protein (kDa)	Expression <i>in vitro</i>		Expression <i>in vivo</i>	
	Iron-sufficient	Iron-deficient	Lung-grown	Chamber-grown
18	++	++	+	+
19	-	-	+	+
31	++	++	+	++
39.5	++	++	+	++
71	+	+++	++	+
77	+	++	++	+
100	+	++	++	+
> 100	-	-	+	+

grown cells (Fig. 1a, lanes 3 and 5). A diffuse band of approximately 52 kDa was present in the lung-grown cells, but not in the *in vitro*-grown cells, whereas a protein of 50 kDa was expressed in *in vitro*-grown cells, but not in lung-grown cells (Fig. 1a, compare lanes 1 and 3, and 4 and 5). The differences between OMP profiles of *in vitro*- and *in vivo*-grown *P. haemolytica* are summarized in Table 1.

The corresponding Western blot, using convalescent antiserum, showed a number of differences in antibody reactivity between the *in vitro*- and lung-grown OMP profiles (Fig. 1b). In neither isolate was there recognition of the 100 kDa iron-regulated protein in lung-grown cells (Fig. 1b, lanes 3 and 5), although this protein was clearly visible in the stained gel (Fig. 1a, lanes 3 and 5). In contrast, the protein was clearly recognized by antibody in both the iron-sufficient and -deficient *in vitro*-grown cells of isolate PH2 (Fig. 1b, lanes 1 and 2). A wide band, possibly representing two discrete proteins, of approximate molecular mass 80 kDa was recognized in the *in vitro*-grown cells, but not in the lung-grown cells of both isolates (Fig. 1b, lanes 3 and 5). The 71 kDa protein was recognized in the lung-grown cells of both isolates, but not in the *in vitro*-grown cells of isolate PH10 (Fig. 1b, lane 4). A protein of approximately 68 kDa was recognized in both lung-grown isolates, but not in the iron-sufficient *in vitro*-grown cells (Fig. 1b, lanes 3 and 5). A further difference was the more pronounced recognition of a protein of approximately 47 kDa in the *in vitro*-grown cells compared to the lung-grown cells. Additional minor differences were also evident between the *in vitro*- and lung-grown cells, including weaker recognition of 50 and 18 kDa proteins in the latter (Fig. 1b, lanes 3 and 5). It was notable that the major 39.5 and 31 kDa proteins could not be clearly identified in Western blots (Fig. 1b). These proteins were either not very immunogenic or not very reactive in Western blots. Similar results were observed in a previous Western blot study (Davies *et al.*, 1994a), in which replicate OMP profiles on nitrocellulose were

stained with amido black for direct comparison with Western blots.

The OMP profiles of *P. haemolytica* isolates PH2 and PH10 obtained from intraperitoneal implant chambers were compared with those of the same isolates obtained from lungs and from cells grown *in vitro* under iron-sufficient or -deficient conditions (Fig. 2a). The reduced expression of the 18 kDa protein, and apparent production of a novel 19 kDa protein, described above in lung-grown cells, was also observed in the chamber-grown cells (Fig. 2a, lanes 3 and 4, and 7 and 8). This similarity in expression of the 18 and 19 kDa proteins, between lung- and chamber-grown cells, confirmed that the chamber-grown cells had indeed adapted to the *in vivo* conditions of the chamber, even though the bacterial population within the chamber was in decline (Davies *et al.*, 1994b). The 19 kDa protein was expressed more in lung-grown cells of isolate PH10 than in those of isolate PH2 (Fig. 2a, lane 7). In contrast to the lung-grown cells, however, the chamber-grown cells did not exhibit such extensive reduction in expression of the 39.5 and 31 kDa major proteins (Fig. 2a, lanes 4 and 8). In addition, there was no enhanced expression of the three iron-regulated proteins in the chamber-grown cells, as there was in the lung-grown cells. In both isolates, there was expression of a minor high-molecular-mass protein of > 100 kDa in chamber-grown cells, but not in *in vitro*-grown cells (Fig. 2a, lanes 4 and 8). A protein of similar molecular mass was also present in lung-grown cells of isolate PH2 (Fig. 2a, lane 3). In isolate PH2, but not in isolate PH10, two proteins of approximate molecular masses 20 and 30 kDa were present in chamber-grown cells, but not in lung-grown or *in vitro*-grown cells (Fig. 2a, lane 4). A diffuse band of approximately 52 kDa was visible in the OMP profiles of chamber-grown cells of isolates PH2 and PH10 (Fig. 2a, lanes 4 and 8) and possibly represented the bound heavy chain of IgG, as described previously (Sutherland *et al.*, 1990). The more intense background staining along the lanes of lung-grown bacteria, particularly in Fig. 2(a), lane 7, was possibly due to contaminating surface-bound material. It was unlikely to have been bacterial capsular or LPS material, since this would also have been present in the *in vitro* profiles. These results are summarized in Table 1.

In convalescent serum, the pattern of antibody binding, in Western blots, to the OMPs of the chamber-grown cells was characterized by the recognition of fewer bands than for *in vitro*- or lung-grown cells (Fig. 2b, lanes 4 and 8). The most noticeable difference between the *in vitro*- and *in vivo*-grown cells was in the recognition of a protein of approximate molecular mass 47 kDa, which was stained intensely in the *in vitro*-grown cells, but poorly stained in the *in vivo*-grown cells (Fig. 2b, compare lanes 1 and 2 with 3 and 4, and 5 and 6 with 7 and 8). In addition, there was no significant recognition of the iron-regulated proteins, especially of the 71 and 100 kDa proteins, in the chamber-grown cells. Differences in the antibody reactivity to OMPs between lung- and chamber-grown cells included the recognition of proteins of 32, 34, 36, 55 and 65 kDa in lung-grown cells, but not in chamber-grown cells (Fig. 2b, lanes 3 and 4, respectively).

Fig. 2. Coomassie-blue-stained SDS-PAGE OMP profiles (a) and Western blot (b) of *in vitro*-, lung- and chamber-grown cells of *P. haemolytica* isolates PH2 (lanes 1–4) and PH10 (lanes 5–8). Bacteria were grown in BHIB (lanes 1 and 5), BHIB supplemented with 20 μ M EDDA (lanes 2 and 6), calf lungs (lanes 3 and 7) and implant chambers (lanes 4 and 8). Size markers are shown in lane 9 in (a).

Fig. 3. Coomassie-blue-stained SDS-PAGE OMP profiles of *P. haemolytica* isolate PH2 grown in BHIB (lane 1), BHIB supplemented with 20 µM EDDA (lane 2), foetal calf serum (lane 3), newborn calf serum (lane 4) and calf lungs (lane 5). Size markers are shown in lane 6.

When the lung- and chamber-grown OMP profiles of isolate PH2 were compared with the profiles of the same isolate grown under various *in vitro* conditions (Davies *et al.*, 1992), it was evident that the *in vivo* profiles closely resembled those of *P. haemolytica* grown in newborn calf serum (Fig. 3). For example, in cells grown in newborn calf serum, there was reduced expression of the 39.5, 31 and 18 kDa proteins, and expression of a high-molecular-mass protein of > 100 kDa (Fig. 3, lane 4, arrowed), as there was in lung-grown bacteria (Fig. 3, lane 5). In addition, in cells grown *in vivo* or in newborn calf serum, expression of the iron-regulated proteins was not significantly enhanced compared with growth *in vitro* in the presence of EDDA (Fig. 3, lane 2). Bound IgG heavy chains (52 kDa) were also evident in cells grown in newborn calf serum, as they were in lung- and chamber-grown cells.

DISCUSSION

The present study examined the expression of OMPs in *P. haemolytica* cells obtained from the natural site of infection, i.e. the lungs, and compared them with those of cells obtained from an intraperitoneal implant chamber and

from *in vitro*-grown cells. Differences were identified between *in vivo*- and *in vitro*-grown *P. haemolytica* that have not been described previously. In addition to enhanced production of the 71, 77 and 100 kDa iron-regulated OMPs in lung-grown cells, there was reduced expression of proteins of 18, 31 and 39.5 kDa and apparent synthesis of a novel 19 kDa protein compared to *in vitro*-grown cells. The reduced expression of the 18 kDa protein in lung-grown cells probably correlated with the reduced expression of a 17 kDa protein observed in a serotype A2 isolate obtained from the pleural fluid of a sheep by Donachie & Gilmour (1988). The enhanced expression of the iron-regulated OMPs in lung-grown cells was not, however, as great as when the cells were grown *in vitro* under iron-restricted conditions.

When the profiles of *in vitro*- and lung-grown cells were examined by Western blotting, with convalescent anti-serum from an experimentally infected calf, some of the differences observed in the SDS-PAGE profiles were not evident. For example, differences in the expression of the 19, 31 and 39.5 kDa proteins were not observed in Western blots. However, there were differences between *in vitro*- and lung-grown cells in the molecular mass range 47–100 kDa. The most significant difference, in both isolates PH2 and PH10, was the lack of recognition of the 100 kDa protein in the lung-grown cells, in contrast to its recognition in the *in vitro*-grown cells, although the protein was clearly expressed in the lung-grown cells as revealed in the stained gels. These findings closely parallel those of Donachie & Gilmour (1988), who were also unable to demonstrate immune recognition of the 100 kDa protein in *in vivo*-grown cells of a serotype A2 isolate from a sheep, although the protein was visible in stained gels. Similarly, these authors were also able to demonstrate immune recognition of the 100 kDa protein in cells grown under iron-restricted *in vitro* growth conditions. These results indicated, therefore, that although the 100 kDa protein is expressed *in vivo*, the protein is not recognized by immune sera in Western blots. The 71 kDa iron-regulated protein was recognized by convalescent serum in the *in vivo*-grown cells, but not in cells grown under iron-sufficient *in vitro* conditions. A 68 kDa protein was also recognized in the lung-grown, but not in the *in vitro*-grown cells, whereas a 47 kDa protein was strongly recognized in the *in vitro*-grown cells, but not in the lung-grown cells. A similar protein seemed to be present in the serotype A2 isolate described by Donachie & Gilmour (1988). In Western blots, these authors described a protein of approximately the same molecular mass in *in vitro*-grown cells, but not in cells grown *in vivo* or in horse serum.

The OMP profiles of the chamber-grown cells were characterized by reduced expression of the 18 kDa protein, and expression of a novel protein of 19 kDa, compared to *in vitro*-grown cells. These characteristics of chamber-grown cells confirmed that the bacteria had indeed adapted to the *in vivo* conditions within the chamber, because these changes also occurred in lung-grown cells. The changes demonstrate that, in some ways at least, the chamber was mimicking the conditions found

within the bovine lung. However, chamber-grown cells differed from the lung-grown cells in a number of ways. Firstly, the 31 and 39.5 kDa proteins were reduced more extensively in the lung-grown cells than in the chamber-grown cells. Secondly, production of the three iron-regulated proteins was not enhanced to the same extent in the chamber-grown cells as in the lung-grown cells.

The findings for the iron-regulated proteins were in disagreement with those of Morck *et al.* (1991), who demonstrated expression of the three iron-regulated proteins in a serotype A1 isolate grown in a peritoneal implant chamber in a rabbit. However, the results were in agreement with those of Confer *et al.* (1992), who were unable to demonstrate expression of these proteins in a serotype A1 isolate grown in a subcutaneous chamber in a calf. It should be pointed out, however, that the profiles described by Confer *et al.* (1992) were whole-cell profiles, and the resolution of individual bands in the high-molecular-mass range was relatively poor. The overall antibody recognition of OMPs from chamber-grown cells was less than that for lung-grown or *in vitro*-grown cells. This was expected because fewer proteins were present in the stained OMP profiles of chamber-grown cells. There was much weaker antibody recognition of a 47 kDa protein in the chamber-grown cells, compared to the *in vitro*-grown cells, as observed with the lung-grown cells. This observation again paralleled the findings in a serotype A2 isolate obtained from the pleural fluid of a sheep (Donachie & Gilmour, 1988). As expected from the OMP profiles, there was no significant recognition of the iron-regulated proteins in the chamber-grown cells. Differences in the antibody recognition of proteins of 32, 36, 55 and 65 kDa between the lung- and chamber-grown cells confirmed the fact that growth conditions were not identical in the two environments.

Finally, when the OMP profiles of the lung- and chamber-grown cells of isolate PH2 were compared with those of cells of the same isolate grown *in vitro* in foetal and newborn calf serum (Davies *et al.*, 1992), a number of common features were evident. In newborn calf serum, for example, there was reduced expression of 18, 31 and 39.5 kDa proteins; this was also observed in both the lung- and the chamber-grown cells. Reduced expression of a 17 kDa protein was also demonstrated, by Donachie & Gilmour (1988), in a serotype A2 isolate grown in horse serum. However, a 19 kDa protein was not apparent in serum-grown cells, as it was in lung- and chamber-grown cells. A high-molecular-mass protein of > 100 kDa was observed in cells grown in newborn calf serum, as well as in chamber-grown cells and possibly in lung-grown cells. In foetal calf serum there was no enhanced production of the 77 or 100 kDa proteins, whereas in newborn calf serum there was no enhanced production of the 71, 77 or 100 kDa proteins (Davies *et al.*, 1992). It should be noted that a protein of molecular mass similar to that of the 100 kDa protein was produced in newborn calf serum, but this protein was immunologically different from the 100 kDa protein produced during other growth conditions (Davies *et al.*, 1992). Similar results were observed in bacteria derived from the implant chambers,

i.e. there was no enhanced production of the iron-regulated proteins. Antibody recognition of the 47 kDa protein was also reduced in cells grown in newborn calf serum (results not shown), as was observed in *in vivo*-grown cells. Binding of IgG heavy chains occurred in both lung- and chamber-grown cells, as well as in cells grown in newborn calf serum, and has been described previously in *in vivo*-grown cells of both *P. haemolytica* (Sutherland *et al.*, 1990) and *Escherichia coli* (Finn *et al.*, 1982). The overall similarity of the OMP profiles of bacteria grown *in vivo* in the lung, and those of bacteria grown *in vitro* in newborn calf serum, including the binding of antibody in both environments, was highly significant. These observations suggest that the *in vivo* environment, during the disease process, may be partly reproduced *in vitro* by growing the bacteria in newborn calf serum. This finding is, perhaps, not surprising because during the disease process, as a result of inflammation, the lung alveoli are flooded with serum-like fluid. Thus, the bacteria within the lung will be growing in a serum-like medium also containing macrophages, neutrophils and various inflammatory products. Conditions in the inflamed lung will also be microaerophilic due to the alveoli becoming filled with exudate. The *in vivo* environment could, perhaps, be even more closely mimicked *in vitro* by adding cellular components to the newborn calf serum, as well as by creating microaerophilic or anaerobic growth conditions.

In summary, this study has demonstrated differences in the OMP profiles, and in the antibody recognition of OMPs, between *P. haemolytica* from the lungs of calves and cells grown *in vitro*. These changes involved proteins in addition to the previously recognized iron-regulated OMPs. The OMP profiles of cells obtained from an intraperitoneal implant chamber showed various changes, some of which were similar to those of the lung-grown cells, others of which were different. In particular, the iron-regulated OMPs were not highly expressed in chamber-grown cells although they were in lung-grown cells. Some of the changes observed in the lung- and chamber-grown cells were also observed in cells grown in newborn calf serum. *In vivo* growth of *P. haemolytica* may be mimicked most accurately *in vitro* by growing the bacteria in newborn calf serum.

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