Selection of Recombinant Antibodies Specific for Pathogenic *Streptococcus suis* by Subtractive Phage Display

ASTRID DE GREEFF,1,2* LOEK VAN ALPHEN,1,3 AND HILDE E. SMITH2

Department of Medical Microbiology, University of Amsterdam, Academic Medical Center, Amsterdam,1 Department of Bacteriology, Institute of Animal Science and Health, 8200 AB Lelystad,2 and Laboratory of Vaccine Development and Immune Mechanisms, RVIM, National Institute of Public Health and the Environment, 3720 BA Bilthoven,3 The Netherlands

Received 6 January 2000/Returned for modification 14 February 2000/Accepted 23 April 2000

A semisynthetic antibody phage display library was used to select recombinant antibodies directed against surface components of a pathogenic strain of *Streptococcus suis* serotype 2 and against extracellular factor (EF), a protein known to be exclusively associated with pathogenic *S. suis* serotype 2 strains. Three distinct monoclonal phage antibodies directed against conformational epitopes of surface protein components of *S. suis* were selected. In addition, three different monoclonal phage antibodies were isolated that recognized EF. To isolate antibody fragments that recognize epitopes specific for a pathogenic *S. suis* serotype 2 strain, compared to a nonpathogenic serotype 2 strain, we applied a subtractive selection procedure. With this procedure, only one distinct phage antibody was found, and it was shown to be directed against EF. This demonstrates the selectivity of the applied procedure and confirms that EF is indeed differentially expressed by pathogenic and nonpathogenic strains. It also shows that EF is a very dominant antigen in phage antibody selections.

Antibody phage display is a very powerful technique for selecting recombinant antibodies from a large library (15, 18, 30). An antibody phage library consists of the variable regions of heavy (VH) and light (VL) chains of human antibodies, which are randomly combined and linked together by a polypeptide linker to form a single-chain fragment (scFv). These scFv's are fused to a minor coat protein of bacteriophage M13, pHIL, resulting in phages displaying antibody fragments. The display of scFv's on a filamentous phage offers the possibility to select phage antibodies without using hybridoma technology. Phage antibodies are selected by panning the library for several rounds on an immobilized antigen. At present, large synthetic libraries are available, which are created from unarranged V gene segments from nonimmunized healthy human donors. These libraries can be used to select antibodies against any given antigen, including foreign antigens, self antigens, non-immunogenic antigens, and toxic antigens (30). In addition, subtractive selection strategies to select for phage antibodies against differentially expressed structures on the surface of different cell types, like thymic cells (25) and human blood cells (16), as well as against proteins differentially expressed on two types of strains of the gram-negative bacterium *Moraxella catarrhalis* (2), have been described.

*Streptococcus suis* is a gram-positive bacterium that can cause severe infections in pigs. Young pigs can suffer from meningitis, septicemia, and arthritis and often do not survive an *S. suis* infection (3, 27). Occasionally, *S. suis* can also cause meningitis in humans (1). Until now, no effective vaccines have been available. Besides, very little is known of *S. suis* in general and its pathogenesis in particular. This makes it difficult to control the disease. So far, 35 capsular serotypes of *S. suis* have been described (6, 7, 12, 19). Worldwide, *S. suis* serotype 2 is the most frequently isolated serotype. Strains of serotype 2 can differ in their virulence: pathogenic, weak-pathogenic, and non-pathogenic strains are recognized (26, 28). Previously, we showed that the expression of two proteins, muramidase-released protein (MRP) and extracellular factor (EF) is strongly associated with pathogenic strains of serotype 2 (28, 29). Therefore, these proteins are considered virulence markers for *S. suis* serotype 2. However, besides MRP and EF, other proteins may be important in the pathogenesis of an *S. suis* infection (5, 8, 9, 13, 14, 28, 29). The use of a phage display library may be of great help in identifying these proteins and in determining the difference between pathogenic and nonpathogenic *S. suis* strains.

Since a considerable number of virulence factors of pathogenic bacteria are either secreted or located on the cell surface, we first tried to select phage antibodies against whole cells of a virulent strain of *S. suis* serotype 2. In addition, phage antibodies were selected against cell-associated structures of a pathogenic strain of *S. suis* after subtraction with a nonpathogenic strain. Three distinct anti-EF phage antibodies, as well as three distinct anti-*S. suis* phage antibodies, were selected. After subtraction, one phage antibody remained, which recognized EF. These data clearly show the successful selection of a phage antibody directed against a protein exclusively expressed by a pathogenic strain of *S. suis* serotype 2 and that EF is a very dominant protein in phage antibody selections.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** Two *Escherichia coli* strains were used in this study as hosts for bacteriophages: TG1 [K-12 Δ(lac-pro) supE thi hsdR515 trpD60 proA Δ(z) thi- leu-2-gal-2 recA thi-2 f' pro-A'] and HB2151 [K-12 Δ(lac-pro) thi-1 F' pro-A' B' lacI-1 lacZAM15 THF]. Both strains were grown on tryptone yeast extract (TYE) plates (17) containing 1% glucose and antibiotics when required. Cultures were grown in 2 X TYE broth (2 X TYE) (17). *S. suis* strain 10 expresses EF and MRP, while *S. suis* strain T15 does not (23, 29). Strain 10 was proven to be pathogenic, while strain T15 was proven to be nonpathogenic in an experimental pig model (23, 29). *S. suis* strain 10cps2EF is an isogenic mutant of *S. suis* strain 10 that is deficient in capsular polysaccharide production (21). *S. suis* strains were grown on Columbia agar blood base plates (code CM331; Oxoid, Ltd., London, United Kingdom), containing 6% (vol/vol) horse blood. Cultures were grown in Todd-Hewitt broth (code CM 189; Oxoid, Ltd.).

**Preparation of antigens.** Stationary-phase *S. suis* cells (100 ml) were centrifuged for 20 min at 2,500 g and washed twice with 100 ml of phosphate-buffered saline (PBS) (0.1 M NaCl, 33 mM Na2HPO4, 17 mM NaH2PO4·2H2O;
pH 7.4). The cells were resuspended in 50 ml of PBS. This suspension was used for coatings, both for the selection procedure and for the enzyme-linked immunoassay (ELISA). The supernatant was collected for use on Western blots. To prepare phage supernatant, each phage-SEPHACRYL S-200 (Pharmacia, Uppsala, Sweden) fraction was used to obtain purified phage antibodies. Griffin.1 is a semisynthetic phage library containing more than 10^8 clones. The library was constructed by recloning the V\textsubscript{H} and V\textsubscript{L} variable regions from the Ig library vectors into the phagemid vector pHEIN2 (11).

**Phage display library.** The Griffin.1 library (a generous gift from Greg Winter, CCLG, Cambridge, U.K.) was used in serial dilutions in 2% MPBS and incubated as described above for the coating of the immunotubes. Before being used, the MoPhAbs did not bind to 3% bovine serum albumin, 2% SDS-polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane by standard procedures (20). The membrane was blocked in Tris-buffered saline (TBS) (50 mM Tris-HCl [pH 7.5], 150 mM NaCl) containing 4% skimmed milk and 0.05% Tween 20 (Blotto) for 1 h. To detect specific antigens, the membranes were incubated with a 1:1 dilution of scFvs in Blotto for at least 90 min. Bound scFvs were visualized with a 1:1,000 dilution of anti-m-cy monoclonal antibody (MAB) (Boehringer) in Blotto-TBS (1:1), followed by an incubation with a 1:1,000 dilution of alkaline phosphatase-conjugated anti-mouse antibody. As a substrate, we used Nitro Blue Tetrazolium (Merck, Darmstadt, Germany)-bromochloroindolyl phosphate (Sigma, St. Louis, Mo.). All washing steps were performed in Blotto-TBS (1:1). A hybridoma-derived MAB directed against EF and convalescent serum raised against S. suis strain 10 in swine were used as positive controls (28).

**Nucleotide sequence analysis.** Nucleotide sequences were determined on a 373A DNA Sequencing System (Applied Biosystems, Warrington, United Kingdom). Samples were prepared with an AB/PRIISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Custom-made sequencing primers were purchased from Life Technologies. Primers used were as follows: Boli 189 (5'-GGTGGAGGCGGTTCACGCGCAGG 9), Boli 189 (5'-GGTGGAGGCGGTTCACGCGCAGG 9), FOR_LINKSEQ (5'-GATTITCGITGATGAGCCGACCGAG TGGCCTCT-3'), and IDSEQL (5'-GAATTTCTTGGATGAGCCGACCGAG TGGCCTCT-3'). Sequencing data were assembled and analyzed with the Lasergene program (DNASTAR).

**RESULTS**

**Selection of phage antibodies.** The Griffin.1 phage library was used to select phage antibodies against purified EF protein. Six rounds of selection were performed on EF. Input phage titers were very uniform, between 5.5 × 10^12 and 4.7 × 10^13. After each round of selection, the number of eluted phages was determined. The results (Table 1) show that phage titers decreased in the first two rounds of selection and rose again in the third round. This indicates antigen-specific phage antibodies were selected and enriched. PoPhAbs derived from the library and the successive selection rounds were subsequently tested for their capacity to bind to purified EF protein in an ELISA (Fig. 1). An increase in the absorbance signal was observed for these phage antibodies obtained after the second selection round. This indicates that antigen-specific PoPhAbs had been selected and enriched after round 2. To confirm this data further, 96 individual, randomly chosen colonies from each selection round were used to prepare PoPhAbs. These MoPhAbs were subsequently tested with an ELISA for their capacity to bind to EF. As shown in Table 2, the number of MoPhAbs which showed specific binding to EF increased after the successive rounds of selection. The MoPhAbs did not bind to 3% bovine serum albumin, 2%
MPBS, Todd-Hewitt medium, or uncoated plates (OD_{600} < 0.150).

Subsequently, the Griffin1 library was used to select phage antibodies against intact S. suis strain 10 cells. Four rounds of selection were performed on intact bacterial cells. Input phage titers were between 5.5 \times 10^{12} and 4.7 \times 10^{13}. After each selection round, the eluted phage titers were determined. The titers decreased in the first two rounds but increased again in the third round (Table 1), indicating that enrichment of antigen-specific phages had taken place.

MoPhAbs derived from the library and the four rounds of selection were tested for their capacity to bind to intact S. suis cells. Figure 1 shows an increase in the absorption signal for MoPhAbs that specifically recognized S. suis. Ninety-six individual, randomly chosen colonies from each selection round were induced to produce MoPhAbs. These MoPhAbs were tested with an ELISA on intact S. suis cells. The number of MoPhAbs that specifically recognized S. suis increased after the successive rounds of selection. The selected MoPhAbs did not bind to 3% bovine serum albumin, 2% MPBS, Todd-Hewitt medium, or uncoated plates (OD_{600} < 0.150). In conclusion, these data indicate that MoPhAbs specific for purified EF protein and intact S. suis strain 10 cells have been selected and enriched.

Diversity of isolated phage antibodies. To determine the diversity among the selected phage antibodies, 96 individual clones obtained after the fifth round of selection against purified EF protein and after the third round of selection against S. suis cells were subjected to PCR. The PCR products were analyzed by restriction enzyme analysis. As a control, 10 randomly chosen clones of the original library were analyzed in the same way. Amplification of a complete scFv fragment will result in a PCR product of about 1 kb. Of 96 clones obtained after selection against EF, 36 showed a PCR product of 1 kb and 11 showed a PCR product of 0.7 to 0.8 kb; for 51 clones, no PCR product was obtained. Of the 96 clones obtained after selection against S. suis cells, 52 showed a PCR product of 1 kb and 24 showed a product of 0.7 to 0.8 kb; for 20 clones, no PCR product was obtained. Moreover, nearly all clones which yielded a PCR product of 1 kb were positive by ELISA. In contrast, clones which did not result in a PCR product were never positive by ELISA. From clones which yielded a PCR product of 0.7 to 0.8 kb, the ELISA signal varied between negative and weakly positive (data not shown). From all 10 clones randomly selected from the library, a 1-kb PCR product was obtained. As expected, these 10 clones showed 10 different BstNI restriction patterns. Among the 36 anti-EF clones, three distinct BstNI restriction patterns were found (E-H1, E-D9, and E-H3) (Fig. 2). Of the anti-EF clones, 92% were of the E-H1 type, 6% of the E-H3 type, and 3% of the E-D9 type. Surprisingly, after PCR and restriction analysis of the sixth selection round, only one clone was found, E-H11 (data not shown). This indicates that the increase of phage titers observed after round 6 was the result of enrichment of a subpopulation of the EF-binding clones. Among the 50 anti-S. suis clones, three other unique BstNI restriction patterns were found (S-A7, S-B1, and S-F7) (Fig. 2). Of the anti-S. suis clones, 63% were of the S-B1 type, 12% of the S-F9 type, and 4% of the S-A7 type.

Immunoblot analysis. The binding specificity of MoPhAb E-H1 was analyzed as representative for anti-EF selected MoPhAbs with a Western blot of culture supernatant of S. suis strain 10. A hybridoma-derived MAb raised against EF and convalescent serum raised against S. suis strain 10 were included as controls. Figure 3 shows that EF, a 110-kDa protein (28), was clearly detected with the MAb as well as with the

### TABLE 1. Phage titers obtained after biopanning on purified EF protein and intact S. suis cells and after a subtractive selection procedure

<table>
<thead>
<tr>
<th>Selection round</th>
<th>Standard selection procedure</th>
<th>Subtractive selection procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EF(^a) Intact cells of S. suis strain 10</td>
<td>Intact cells of S. suis strain 10</td>
</tr>
<tr>
<td>0</td>
<td>4.7 \times 10^{13} 4.7 \times 10^{13}</td>
<td>5.0 \times 10^{12} 5.0 \times 10^{12}</td>
</tr>
<tr>
<td>1</td>
<td>3.0 \times 10^{6} 3.5 \times 10^{6}</td>
<td>1.0 \times 10^{5} 1.5 \times 10^{5}</td>
</tr>
<tr>
<td>2</td>
<td>3.5 \times 10^{3} 6.5 \times 10^{3}</td>
<td>4.5 \times 10^{3} 5.0 \times 10^{4}</td>
</tr>
<tr>
<td>3</td>
<td>2.0 \times 10^{6} 5.3 \times 10^{6}</td>
<td>4.0 \times 10^{4} 7.0 \times 10^{5}</td>
</tr>
<tr>
<td>4</td>
<td>2.0 \times 10^{6} 1.5 \times 10^{6}</td>
<td>5.0 \times 10^{3} 1.0 \times 10^{7}</td>
</tr>
<tr>
<td>5</td>
<td>4.1 \times 10^{6}</td>
<td>3.0 \times 10^{3} 8.3 \times 10^{7}</td>
</tr>
<tr>
<td>6</td>
<td>1.0 \times 10^{8}</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Purified protein. 
\(^{b}\) Subtraction with S. suis strain T15.
convalescent serum. A protein band of the same size was detected by using the scFv preparation of the selected MoPhAb, E-H1. These results show that E-H1 was indeed specifically directed against EF. Therefore, the antibody phage display is a fast and efficient method to select MoPhAbs against purified EF.

To characterize the MoPhAbs selected against intact S. suis cells, Western blots containing either culture supernatant or protoplast supernatant of S. suis strain 10 were incubated with MoPhAbs or scFvs of the three S. suis-specific clones. No specific band was detected. One possible explanation for this is that the selected MoPhAbs are directed against cytoplasmic components and that these components were not present on the blots used. However, because intact S. suis cells were used in the selection procedure, it is not very likely that the antibodies react with cytoplasmic proteins. Since strain 10 of S. suis serotype 2 is highly encapsulated, phage antibodies selected against intact S. suis cells may be directed against capsular polysaccharides. To test this possibility, we determined the capacity of the three anti-S. suis MoPhAbs to bind to strain 10cpsΔEF, an isogenic mutant of strain 10 deficient in capsular polysaccharide production (20). All three phage antibodies bound to the capsular mutant strain as efficiently as to the wild-type strain (OD_{600} > 0.350), thereby excluding the possibility that the phage antibodies are directed against capsular polysaccharide components. A further possibility is that the selected MoPhAbs recognize conformational epitopes and therefore do not recognize the denatured and reduced antigens on the Western blot. Protoplast supernatant was spotted on a blot under denaturing and nondenaturing conditions, and the dot blot was subsequently incubated with MoPhAbs prepared from clones S-A7, S-B1, and S-F9. Figure 4 shows that nondenatured proteins present in protoplast supernatant were recognized by the three selected MoPhAbs, whereas the denatured proteins were not recognized, except for clone S-F9, which showed a weak reaction with denatured proteins. This result shows that the MoPhAbs were directed against cell surface proteins of S. suis and that they recognize conformational epitopes.

### Table 2: Specificity of MoPhAbs selected against indicated antigens as determined by ELISA

<table>
<thead>
<tr>
<th>Selection round</th>
<th>No. (%)&lt;sup&gt;a&lt;/sup&gt; of MoPhAbs which bind to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EF&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>0 (&lt;0.2)</td>
</tr>
<tr>
<td>1</td>
<td>0 (&lt;0.2)</td>
</tr>
<tr>
<td>2</td>
<td>0 (&lt;0.2)</td>
</tr>
<tr>
<td>3</td>
<td>3 (3)</td>
</tr>
<tr>
<td>4</td>
<td>22 (23)</td>
</tr>
<tr>
<td>5</td>
<td>36 (38)</td>
</tr>
<tr>
<td>6</td>
<td>45 (47)</td>
</tr>
</tbody>
</table>

<sup>a</sup>n = 96.

<sup>b</sup>Purified protein.

<sup>c</sup>After subtraction with S. suis strain T15.

![FIG. 2. BstNI fingerprints of the inserts of phages selected against purified EF protein and S. suis cells. Individual clones were subjected to PCR and restricted with BstNI. Lane 1, EF-specific clone E-H1; lane 2, EF-specific clone E-D9; lane 3, EF-specific clone E-H3; lane 4, S. suis-specific clone S-A7; lane 5, S. suis-specific clone S-B1; lane 6, S. suis-specific clone S-F9. The size of products is indicated in base pairs.](image)

![FIG. 3. Western blot analysis of the culture supernatant of S. suis strain 10, probed with a classical MAb raised against EF (lane 1); convalescent serum raised against S. suis (lane 2); and scFvs of clone E-H1 (lane 3). Arrowhead, 110-kDa EF protein.](image)

![FIG. 4. Dot blot analysis of the protoplast supernatant of strain 10, probed with three anti-S. suis MoPhAbs selected against S. suis cells. (A) protoplast supernatant in TBS; (B) protoplast supernatant in SDS loading buffer; (C) native loading buffer. Proteins were either incubated with S-A7 (lane 1), S-B1 (lane 2), or S-F9 (lane 3). Convalescent serum raised against S. suis strain 10 was used as a positive control (lane 4).](image)
Subtractive selection on S. suis strain 10. To identify proteins exclusively expressed by a pathogenic S. suis serotype 2 strain, phage antibodies were selected against intact cells and against protoplast supernatant of the pathogenic S. suis strain 10, after subtraction with the nonpathogenic S. suis strain T15, lacking protein EF. Five rounds of selection were performed on both antigens. Input phage titers for both selections were fairly constant, between $5 \times 10^{12}$ and $3.8 \times 10^{13}$.

For selection on intact cells of S. suis strain 10, phage titers decreased in the first four rounds of selection and increased slightly after the fifth round (Table 1). To test the specificity of the selected phage antibodies, 96 randomly chosen colonies from the library and from selection rounds four and five were induced to produce MoPhAbs. The binding of these MoPhAbs to proteins present on intact cells of S. suis strain 10 was determined by an ELISA. After five rounds of selection, three positive clones were found (Table 2).

For selection on protoplast supernatant of S. suis strain 10, phage titers decreased in the first two rounds of selection and increased again after the fourth round of selection (Table 1), indicating enrichment of antigen-binding phages. To test the specificity of the selected phage antibodies, 96 randomly chosen colonies from each selection round were induced to produce MoPhAbs. The binding of the MoPhAbs to proteins present in the protoplast supernatant of S. suis strain 10 was determined. The number of MoPhAbs which showed specific binding to the protoplast supernatant of S. suis strain 10 in ELISA increased in the successive selection rounds (Table 2), indicating enrichment of S. suis-specific clones.

Clones that were positive in the monoclonal phage ELISAs on intact cells or on protoplast supernatant of S. suis strain 10 were subsequently tested by PCR and fingerprint analysis. Ninety-seven of these clones, including the three clones selected on intact S. suis cells, showed a PCR product of 1 kb. Moreover, all 97 clones showed an identical BstNI restriction pattern. Surprisingly, this pattern was identical to the pattern of clone E-H1, indicating that clone E-H1 and the subtractive clone (Sub-B3) are identical. Clones E-H1 and Sub-B3 were further characterized by AvaII fingerprints. Figure 5 shows that the AvaII restriction patterns of E-H1 and Sub-B3 were identical. These data strongly indicate that the phage antibodies selected by the subtractive procedure are similar to those of E-H1 and therefore directed against EF. Since strains 10 and T15 are known to differ in the expression of EF (28, 29), these data suggest that the subtractive selection procedure has succeeded. This was further confirmed by Western blots of culture supernatants of S. suis strains 10 and T15 that were incubated with scFvs derived from Sub-B3. As control, a MAb raised against EF was used. The 110-kDa EF protein was recognized by Sub-B3 and the MAb against EF in culture supernatant of strain 10 but not in culture supernatant of strain T15 (Fig. 6).

Nucleotide sequence analysis of V regions of selected MoPhAbs. The nucleotide sequence of all seven selected MoPhAbs was determined and analyzed by use of the V-BASE sequence directory described by Tomlinson et al. (24) (Table 3). As expected, based on their identical restriction patterns, clones E-H1 and Sub-B3, both recognizing EF but selected on different antigens, used the same V genes. Remarkably, however, the CDR3 region of both clones was different, both in amino acid composition and in charge. Both the V genes and the CDR3 sequences of the other five clones were very variable, as was expected based on the large differences between the restriction patterns of those clones.

## DISCUSSION

A semisynthetic antibody phage library was used to select recombinant antibodies directed against surface components of a pathogenic strain of S. suis serotype 2, including EF, a protein known to be exclusively associated with pathogenic S. suis serotype 2, including EF, a protein known to be exclusively associated with pathogenic S. suis serotype 2 strains (28, 29). Using purified EF protein as an antigen, three unique anti-EF phage antibodies were selected, probably directed against dif-
different linear epitopes of EF. On a Western blot, anti-EF clone E-H1 recognized EF as efficiently as a hybridoma-derived MAb raised against EF. This clearly shows that selection on purified EF protein yielded MoPhAbs specific for EF.

Using intact S. suis serotype 2 cells as an antigen, three distinct phage antibodies were selected. These MoPhAbs recognized proteins present in the protoplast supernatant fraction of S. suis strain 10, indicating that the MoPhAbs are directed against proteins present on the cell surface of S. suis. The phages reacted with nondenatured proteins of encapsulated S. suis and a nonencapsulated mutant, indicating that the MoPhAbs were directed against conformational protein epitopes.

As determined by PCR, only 50% of the selected clones contained a full-sized insert of 1 kb. Twenty-five percent of the clones showed a small-sized product of about 0.7 to 0.8 kb, and 25% did not contain an insert at all. Similar results were described previously by de Bruin et al. (4). These authors also described the selection of phages containing small-sized inserts after using the Griffin I library. In addition, they showed that these phages were already present in the original library and represented a few phages that did not obtain a V_{H} region during the construction of the library. In mixed cultures, phages containing small-sized inserts tended to overgrow the phages containing full-sized inserts (4).

With a pathogenic and a nonpathogenic strain in a subtractive selection procedure, one distinct phage antibody (Sub-B3) was selected that seemed to be identical to the phage antibody selected on EF-coated immunotubes (E-H1), as determined by PCR and fingerprint analysis. Nucleotide sequence analysis confirmed that both clones used the same V genes. Remarkably, clones E-H1 and Sub-B3 used different CDR3 regions. Therefore, Sub-B3 may bind to EF with a different affinity or to another epitope than E-H1. Whether this is true remains to be determined. Sub-B3 was shown to recognize EF on a Western blot. This clone was found both after subtractive selection on intact S. suis cells and on protoplast supernatant of S. suis. Since no EF-recognition scFv’s were selected when the library was panned on intact S. suis cells without subtraction, it can be concluded that the subtractive selection procedure was successful.

Table 3. Deduced amino acid sequences of heavy-chain CDR3 and usage of V_{H} and V_{L} genes by seven MoPhAbs selected against EF or S. suis.

<table>
<thead>
<tr>
<th>Clone</th>
<th>CDR3 sequence</th>
<th>Germ line segment*</th>
<th>V_{H}</th>
<th>V_{L}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-H1</td>
<td>NKEMP</td>
<td>DP49</td>
<td>Va3</td>
<td></td>
</tr>
<tr>
<td>E-D9</td>
<td>LRETS</td>
<td>DP23</td>
<td>VaII</td>
<td></td>
</tr>
<tr>
<td>E-H3</td>
<td>PALPFWNT</td>
<td>DP73</td>
<td>VxI</td>
<td></td>
</tr>
<tr>
<td>S-A7</td>
<td>NYVNAPSR</td>
<td>DP15</td>
<td>VxI</td>
<td></td>
</tr>
<tr>
<td>S-B1</td>
<td>LGPLG</td>
<td>DP44</td>
<td>VA1</td>
<td></td>
</tr>
<tr>
<td>S-F9</td>
<td>GTRNP</td>
<td>DP47</td>
<td>VA1</td>
<td></td>
</tr>
<tr>
<td>Sub-B3</td>
<td>ANSNRKF</td>
<td>DP49</td>
<td>VA3</td>
<td></td>
</tr>
</tbody>
</table>

* Assignment of germ line V_{H} and V_{L} segments is according to the V-BASE sequence directory described by Tomlinson et al. (24).

To select phages specific for proteins other than EF exclusively expressed by the pathogenic S. suis serotype 2 strains, an isogenic mutant of pathogenic strain 10 deficient in the expression of EF may be helpful.

REFERENCES


