Synthesis in Escherichia coli and Immunological Characterization of a Polypeptide Containing the Cleavage Sites Associated with Trypsin Enhancement of Rotavirus SA11 Infectivity

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SUMMARY

About 45% of the rotavirus SA11 VP3 gene was inserted into a thermoinducible expression plasmid under the control of phage lambda P1 promoter. The primary translation product predicted on the basis of the plasmid construction was a hybrid protein in which the 98 amino-terminal amino acids of phage MS2 polymerase were followed by amino acids 42 to 387 of the VP3 protein, which included the region containing the cleavage sites associated with trypsin enhancement of infectivity. On induction, a polypeptide that had the expected mol. wt. and contained VP3-related amino acid sequences as judged by immunological criteria, was synthesized to a level representing about 15% of the total bacterial protein. When a bacterial lysate enriched for the fusion polypeptide was injected into mice, it induced antibodies which inhibited haemagglutination and neutralized SA11 rotavirus infectivity.

INTRODUCTION

In developing countries, acute gastroenteritis is one of the leading causes of morbidity and mortality in children under the age of 5, with rotaviruses being probably the single most important aetiological agent (Flewett & Woode, 1978; Holmes, 1983). These viruses, which also cause diarrhoea in the young of many other mammals and birds, were first reported in association with human infantile gastroenteritis by Bishop et al. (1973). Since then, knowledge of rotaviruses has accumulated very quickly because of their great medical and economic importance.

The virions are composed of 11 segments of double-stranded RNA (dsRNA) contained in a double layer of proteins (Rodger & Holmes, 1979). In the simian rotavirus SA11, this double capsid is composed of five polypeptide species (Ericson et al., 1982; Espejo et al., 1981), three of which form the inner capsid, while the other two, a protein with a relative molecular mass (Mr) of 88000 (VP3) (Espejo et al., 1981; Estes et al., 1981) and a glycoprotein of Mr 37000 (VP7) (Arias et al., 1982a; Ericson et al., 1983), are in the surface layer of the virus. These two outer layer proteins, the products of genes 4 and 9, respectively (Arias et al., 1984; Both et al., 1983; López et al., 1985; Mason et al., 1983), have been shown to induce neutralizing antibodies (Bastardo et al., 1981; Greenberg et al., 1983b; Matsuno & Inouye, 1983). In addition, VP3 has been identified as the virus haemagglutinin (Kalica et al., 1983) and the product of the gene limiting the growth of fastidious rotavirus strains in cell culture (Greenberg et al., 1983a).

The infectivity of rotaviruses in cell culture can be increased by, and probably is dependent on, trypsin treatment (Clark et al., 1981; Estes et al., 1981; Matsuno et al., 1977). This proteolytic activation of the virus produces a specific cleavage of the outer layer protein VP3, generating two cleavage products, VP5 (Mr 60000) and VP8 (Mr 28000) [the nomenclature used in Espejo et al. (1981) will be followed] (Espejo et al., 1981; Estes et al., 1981; López et al., 1985).

Monoclonal antibodies have been produced against VP3 of simian rotavirus SA11 and rotavirus NCDV, a heterotypic bovine isolate (Ikegami & Akatani, 1984). In their work, all the
isolated neutralizing antibodies reacted with the smallest cleavage product, VP8. One antibody neutralized both strains of rotavirus and, when incubated with the viruses before trypsin treatment, prevented the cleavage of VP3. This suggests that the cleavage region or a site near it may be able to elicit cross-neutralizing antibodies.

Two other studies have described the isolation of monoclonal neutralizing antibodies directed against VP3 of rotavirus, which also were capable of neutralizing heterotypic strains (Offit et al., 1986; Taniguchi et al., 1985). One of these studies demonstrated that these monoclonal antibodies passively protected suckling mice when challenged with rotaviruses belonging to three different serotypes (Offit et al., 1986).

The induction of cross-serotype neutralizing and protecting antibodies by VP3 makes this viral protein, and especially the cleavage region, a very attractive candidate for use as a vaccine produced by DNA recombinant methods. Here, we report the synthesis in _Escherichia coli_ of about 45% of the length of the VP3 protein, including the region containing the cleavage sites associated with trypsin enhancement of rotavirus infectivity. This bacterial product, designated VP8′, was able to induce haemagglutination inhibiting as well as neutralizing antibodies to SA11 rotavirus.

**METHODS**

*Viruses and cells.* The rotavirus used throughout this study was simian rotavirus SA11, obtained originally from H. H. Malherbe (University of Texas) in 1977. SA11 virus was grown in MA104 cells and purified as described by Espeseth et al. (1981).

*Bacterial strains and plasmids. _E. coli._* strain RR14 [F−, hsdS20(r−, m−1), ara-14, proA2, lacY1, galK2, rpsL20(Sm−), xyl-5, met-l, supE44, Δ], was obtained from A. Covarrubias (University of Mexico). This strain synthesizes repressor for the _λ_ P, promoter from a chromosomal _cl_ gene, thereby maintaining repression of the transcription from this promoter. _E. coli_ M5219 (Remaut et al., 1981) is the designation for the strain M72, lacZam, trpam, Sm′, (Δbio252 cI857 ΔH1). This strain carries a temperature-sensitive repressor gene (c1857) for the _λ_ P, promoter, and hence expression from this promoter can be controlled by temperature. Transcription at 28 °C is below detectable limits; a fully induced state is obtained at 42 °C. Plasmids pPLc24 and pSR4-2 have been reported earlier (López et al., 1985; Remaut et al., 1981).

*Recombinant DNA.* Plasmid DNA manipulations were carried out essentially as summarized by Maniatis et al. (1982). Restriction endonucleases, T4 DNA ligase, and the large fragment of _E. coli_ DNA polymerase I were purchased from New England Biolabs. Bacterial alkaline phosphatase was from Bethesda Research Laboratories.

*Nucleic acid sequence determination.* The nucleotide sequence at the junction region of the MS2 polymerase gene and the VP8′ gene in pUMA480ML was determined, starting from the _BamH1_ site of the plasmid, by the method of Maxam & Gilbert (1980). The enzyme used for the second digestion was _PstI_ which cleaves a site in the β-lactamase gene of the plasmid.

*Growth and induction of bacteria.* Bacteria were grown in the maximal induction medium described by Mott et al. (1985). After growth at 28 °C to OD600 = 1.0, plasmid-containing bacteria were heat-induced by addition of an equal volume of medium preheated to 65 °C, with subsequent incubation at 42 °C for the times indicated in the figure legends.

*Polyacrylamide gel electrophoresis.* Samples were analysed in SDS-polyacrylamide gels (SDS–PAGE; either 10%:0.26% or 15%:0.4% acrylamide:bisacrylamide, as indicated in the figure legends) following the method of Laemmli (1970). Bacterial proteins were solubilized by treatment with Laemmli’s sample buffer in a boiling water bath for 3 min and, before loading the gel, the samples were centrifuged at 12000 g for 5 min to remove insoluble material. To estimate the percentage of the hybrid protein in a sample, the gel was stained with Coomassie Brilliant Blue and scanned with a densitometer (Arias et al., 1982b).

*Immunoblot analysis.* Bacterial proteins were separated by electrophoresis and transferred to nitrocellulose paper (BA85, pore size 0.45 μm; Schleicher & Schuell) essentially by the method of Tsang et al. (1983). After blocking non-specific binding sites on the filter with four changes (15 min each) of TNTG (10 mm-Tris–HCl pH 7.4, 0.9% NaCl, 0.3% w/v gelatin), the strips were incubated with a 500-fold dilution of guinea-pig anti-rotavirus SA11 hyperimmune serum (neutralizing titre of 1:30000) for 60 min at 37 °C. Filters were then washed four times with TNTG (10 min each) and incubated with 0.5 μCi of 125I-labelled Protein A per ml in TNTG for 60 min at 37 °C. Unbound Protein A was removed by washing the filters three times. Filters were dried and autoradiographed.

*Enrichment of the hybrid protein.* The chimeric protein was obtained, enriched about threefold, in the pellet after low speed centrifugation of a bacterial lysate produced as described by Kleid et al. (1981). In brief, recombinant bacteria were collected by centrifugation, resuspended in TEN (50 mm-Tris–HCl pH 7.5; 0.5 mm-EDTA, 93 mm NaCl), treated on ice for 15 min with lysozyme (1 mg/ml), and NP40 was added to give a final concentration of 0.2%. After 10 min, NaCl and MgCl2 were added to give final concentrations of 0.9 M and 7.2 mm, respectively.
Expression of rotavirus SA11 gene 4 in E. coli

This mixture was stirred on ice with 2 µg/ml of DNase I for 60 min. Insoluble MS2-VP8’ protein was recovered from the lysate by centrifugation at 4000 g for 15 min and then resuspended and washed three times with TEN.

Immunization schedule. Male BALB/c mice from 7 to 9 weeks of age were screened on day 0 for neutralizing antibodies for SA11 rotavirus and for haemagglutination inhibiting antibodies, as described below. All preimmune sera had haemagglutination inhibiting antibody titres of 1:200. Those mice whose preimmune sera at dilutions of 1:50 failed to neutralize SA11 were divided into three groups according to the immunogen they received. Four mice (group I) were inoculated with bacterial proteins from E. coli M5219 containing plasmid pUMA481ML, which had been enriched for the hybrid polypeptide MS2-VP8’ as described above. Each mouse received 100 µg of protein per injection, of which about 30 µg corresponded to VP3 sequences. Two mice (group II) received per injection 100 µg of bacterial proteins from untransformed E. coli M5219. Two mice (group III) were injected each time with 50 µg of purified SA11 virus (about 5 µg corresponded to VP3 sequences). All mice were injected subcutaneously with 200 µl of an emulsion of the respective antigen and adjuvant. Freund’s complete adjuvant was used for the first immunization; for subsequent injections, incomplete adjuvant was used. Each of the three groups received five injections, on days 0, 14, 29, 44 and 59. Mice were bled on day 66. Each serum was heat-inactivated at 56 °C for 1 h and stored at 4 °C.

Neutralizing antibody assay. Neutralizing antibody titres in mouse sera were measured by the procedure described by Shaw et al. (1985), with some modifications. In brief, equal volumes of SA11 virus (a dilution that produced about 500 stainable cells per well) pretreated with trypsin (Difco 1:250, final concentration 10 µg/ml) and twofold serial dilutions of mouse sera were mixed and allowed to react for 1 h at 37 °C. Each mixture (50 µl) was added to MA104 cell monolayers in a 96-well tissue culture plate. After adsorption for 1 h at 37 °C, each inoculum was removed and 200 µl of minimal essential medium was added to each well. The cells were then incubated for 12 h at 37 °C. After this time, the cells were washed twice with phosphate-buffered saline (PBS) and fixed for 15 min with cold 80% acetone in PBS. The cells were then washed with PBS and incubated with guinea-pig anti-SA11 serum (neutralizing titre of 1:30000) diluted 600-fold in PBS, for 1 h at 37 °C. After two washings with PBS, Protein A–horseradish peroxidase (Amersham) diluted 2500-fold in PBS was added, and the mixture was incubated for 2 h at 37 °C. The cells were then washed with PBS, the peroxidase sustrate 3-amino-9-ethylcarbazole (Sigma) was added and the mixture was incubated in the dark for 10 min at 37 °C. Finally, the plates were washed four times with water, air-dried, and examined by light microscopy. The titre of neutralizing antibody in a serum sample was defined as the highest serum dilution at which a reduction of at least 60% in the number of infected cells was observed as compared with controls in which PBS had been used instead of serum.

Haemagglutination inhibition test. Each serum sample, serially diluted in 0·2% (w/v) rabbit serum albumin (RSA) in PBS, was mixed with an equal volume of this diluent containing four haemagglutinating units of SA11 virus. To 50 µl of this mixture, 50 µl of 0·4% human type O erythrocytes in 0·2% RSA was added. The inhibition of haemagglutination was scored after a 90 min incubation at room temperature.

RESULTS

Construction of a plasmid for expression of rotavirus SA11 gene 4(5')

The viral cDNA (in pSR4-2) used for the construction of the recombinant expression plasmid was designated gene 4(5'), since it contained sequences of the 5' region of rotavirus SA11 gene 4 (see Fig. 1 a). The polypeptide product it encoded was designated VP8’. The strategy used for the construction of this expression plasmid is depicted in Fig. 1 (b).

The acceptor plasmid, pPPlc24, contained the λ P L promoter followed by a DNA fragment containing the ribosome-binding site and part of the MS2 polymerase gene. Immediately downstream from this DNA fragment there was a linker sequence containing one restriction site for BamHI and one for HindIII. Any coding DNA inserted into one of these two sites should be expressed as a chimera with the MS2 gene, under the control of the λ P L promoter.

For the construction of the expression vector, plasmid pSR4-2 was digested with Hhal, and the resulting 1039 bp DNA fragment, containing the SA11 gene 4(5’), was ligated into the HindIII site of pPPlc24. To make the ends of these two DNA fragments suitable for ligation, they were made flush. The Hhal-generated 3'-protruding ends of the insert were made blunt by using the exonuclease activity of the Klenow enzyme and the 3'-recessive ends of the vector were filled in by the polymerase activity of the same enzyme. The resulting plasmid, designated pUMA480ML, was sequenced at the junction region by the method of Maxam & Gilbert (1980). The sequence obtained showed that the gene 4(5’) information was not in frame with the MS2 sequences (data not shown).
Fig. 1. Construction of a vector expressing part of the rotavirus SA11 gene 4. (a) Relative position of the viral cDNA insert in plasmid pSR4-2 with respect to the full length gene 4. This cDNA contains nucleotide (nt) 19 to 1251 of the coding strand of the gene (see text). The arrow indicates the region encoding the trypsin cleavage sites associated with the enhancement of rotavirus SA11 infectivity. (b) Steps involved in the construction of the expression plasmid pUMA481ML.

To correct the frame of the hybrid gene in pUMA480ML, the plasmid was digested with BamHI, its ends were filled in with the Klenow fragment, and then the plasmid was re-ligated to itself. This treatment caused a four nucleotide insertion at the junction region which left the two coding DNA fragments in a continuous reading frame (see Fig. 2b). This plasmid was designated pUMA481ML.

From the known nucleotide sequences of the MS2 genome (Fiers et al., 1976), of the rotavirus SA11 gene 4(5') (López et al., 1985) and of pBR322 (Sutcliffe, 1978), the predicted continuous
Expression of rotavirus SA11 gene 4 in E. coli

Fig. 2. (a) Schematic representation of the expected MS2–VP8' hybrid polypeptide directed by pUMA481ML. The asterisk represents the cleavage sites [amino acids (aa) 241 and 247 of VP3, see Discussion] associated with trypsin enhancement of rotavirus SA11 infectivity. (b) Nucleotide sequence and the encoded amino acid sequence of the junction regions, boxed in (a), predicted on the basis of plasmid construction. The numbers above the amino acids represent the position of that amino acid in the original protein. The X stands for a termination codon.

reading frame would start at the initiating AUG of the MS2 polymerase gene and would finish in the pBR322 sequence at a terminating UGA codon located 45 nucleotides downstream of gene 4(5'). The expected fusion product would consist of 98 amino acids of the MS2 polymerase, six amino acids coded by the vector at the MS2–VP8' junction region, 346 amino acids of VP3 (amino acids 42 to 387, see Discussion), three linker-encoded amino acids and 12 amino acids of pBR322 (see Fig. 2).

All the constructed chimeric plasmids were transformed into E. coli RR1(λ) and, after being characterized, were transferred into E. coli M5219, allowing temperature-dependent controlled expression of the λ P_L promoter.

Expression of rotavirus SA11 gene 4(5')

Transcription from the P_L promoter on the plasmid can be turned on by shifting the growing culture from 28 °C to 42 °C. From SDS–PAGE analysis of the total proteins of temperature-induced E. coli M5219 containing plasmid pUMA481ML, it was evident that at 12 h post-induction several proteins that were not synthesized by the non-induced recombinant bacteria were now present (Fig. 3a, lanes 1 and 2). At this time of induction, a polypeptide of M_r 52000 became the major bacterial product. After scanning the gel with a densitometer, the 52000 M_r protein was calculated to constitute approximately 15% of the total cellular protein. The M_r of this major product was very close to 50 631, the expected value for the hybrid MS2–VP8' protein based on the plasmid construction.

It is well known that upon heat shock some E. coli genes are turned on and others are turned off (Cowing et al., 1985; Schlesinger et al., 1982). Therefore, after 12 h of heat induction, E. coli M5219 cells containing either pPLc24 (Fig. 3b, lane 1) or pUMA481ML (Fig. 3b, lane 2) were lysed and the total proteins were separated by SDS–PAGE. From the Coomassie Brilliant Blue staining patterns, the only appreciable differences between the proteins synthesized in these two strains were the presence of the 52000 M_r protein in the cells containing plasmid pUMA481ML and the presence of a 12000 M_r polypeptide in those containing plasmid pPLc24. These two polypeptides presumably represented the hybrid MS2–VP8' protein and the product of the portion of the MS2 polymerase gene carried by pPLc24, respectively.
Fig. 3. Synthesis of the fusion protein by plasmid-bearing cells. (a) Analysis in a 10% SDS-polyacrylamide gel of total cell proteins from *E. coli* strain M5219 containing plasmid pUMA481ML, before (lane 1) and after (lane 2) 12 h of temperature induction. (b) Analysis in a 15% SDS-polyacrylamide gel of total cell proteins from *E. coli* M5219 containing either pPLc24 (lane 1) or pUMA481ML (lane 2) after 12 h of heat induction. The proteins were visualized by Coomassie Brilliant Blue staining. The arrow indicates the putative MS2-VP8' hybrid polypeptide. The Mr markers ($10^3$) are 200, myosin (H chain); 97.4, phosphorylase B; 68, bovine serum albumin; 43, ovalbumin; 25.7, α-chymotrypsinogen. The positions of the markers indicated apply to the 10% SDS-polyacrylamide gel.

The 52000 Mr protein was highly insoluble since it was found in the pellet that sedimented during low speed centrifugation of the bacterial cell lysate (see Methods). SDS-polyacrylamide gel analysis of the proteins in the pellet showed that the 52000 Mr protein had been enriched and represented about 50% of the total proteins in the pellet (Fig. 4).

Immunological characterization of the MS2-VP8' polypeptide

To investigate whether the 52000 Mr protein contained rotavirus SA11-related antigenic determinants, cultures of *E. coli* M5219 containing either pUMA480ML or pUMA481ML were heat-induced overnight. The proteins in the bacterial lysates were separated by SDS-PAGE (Fig. 5, lanes a to d) and were either stained with Coomassie Brilliant Blue (lanes a and b) or transferred to nitrocellulose paper (lanes c and d). The proteins in the nitrocellulose were first incubated with anti-rotavirus SA11 serum, and then probed with $^{125}$I-labelled Protein A. The
Expression of rotavirus SA11 gene 4 in E. coli

Fig. 4. Analysis in a 10% SDS-polyacrylamide gel of the hybrid protein MS2-VP8' enriched by low speed centrifugation. (a) Total cell proteins from E. coli M5219 containing plasmid pUMA481ML after 12 h of temperature induction. (b) Proteins in the pellet after low speed centrifugation of the sample in lane (a). (c) Proteins in the supernatant after low speed centrifugation of the sample in lane (a). The arrow indicates the MS2-VP8' polypeptide.

Fig. 5. Immunoblot analysis of the proteins synthesized by plasmid-bearing bacteria. Total cell lysates from heat-induced (12 h) E. coli M5219 containing either plasmid pUMA480ML (lanes a and c) or pUMA481ML (lanes b and d) were separated in a 10% polyacrylamide gel and were either stained with Coomassie Brilliant Blue (lanes a and b) or transferred to nitrocellulose paper (lanes c and d). The transferred proteins were incubated with a 500-fold dilution of anti-rotavirus SA11 serum, and the bound antibody was identified with iodinated staphylococcal Protein A. The arrow indicates the putative MS2-VP8' hybrid protein.

The autoradiograph of this immunoblot (Fig. 5, lanes c and d) showed that the anti-rotavirus serum reacted specifically with the 52000 Mr protein which was synthesized only by bacteria containing pUMA481ML (lane d). A minor, lower Mr band also reacted with this serum. However, this band was also present in the bacteria harbouring pUMA480ML, the vector carrying gene 4(5') out of frame with respect to the MS2 information (lane c). These results suggest that the 52000 Mr polypeptide was indeed the expected MS2-VP8' fusion product.
Table 1. Induction of neutralizing and haemagglutination inhibiting antibodies by the hybrid polypeptide MS2-VP8' synthesized in E. coli

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Immunogen</th>
<th>Neutralizing antibody titre of sera taken on day*</th>
<th>Haemagglutination inhibiting antibody titre of sera taken on day†</th>
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<tr>
<td>Group I</td>
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<tr>
<td>1</td>
<td>MS2-VP8'</td>
<td>1:50</td>
<td>1:200</td>
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<tr>
<td>2</td>
<td>MS2-VP8'</td>
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<td>3</td>
<td>MS2-VP8'</td>
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<td>4</td>
<td>MS2-VP8'</td>
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<td>Group II</td>
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<tr>
<td>5</td>
<td>Control‡</td>
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<tr>
<td>6</td>
<td>Control</td>
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<tr>
<td>8</td>
<td>SA11 virus</td>
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*Titres are expressed as the reciprocal of the highest dilution of the serum that neutralized at least 60% of the immunoperoxidase foci in the assay.
†Titres are expressed as the reciprocal of the highest dilution of the serum that completely inhibited the haemagglutination of human type O erythrocytes by the SA11 virus.
‡Bacterial proteins from host E. coli M5219 treated in the same way as those from the plasmid-carrying bacteria (see Methods).

To test the capability of the MS2-VP8' polypeptide to elicit the induction of neutralizing antibodies to SA11 virus, a bacterial lysate enriched for the hybrid protein, as described above, was injected into mice. The mice which received this immunogen did develop neutralizing antibodies to the virus to a mean titre of 1:1200, whereas sera from control animals which had been immunized with a bacterial lysate of an E. coli strain that did not have the recombinant plasmid did not show neutralizing activity (Table 1).

When mice were immunized with purified SA11 virions, the neutralizing antibody titres obtained were 20 times higher than those obtained by injection of the recombinant product. However, the titres of haemagglutination inhibiting antibodies induced by either of these immunogens were quite comparable, representing in both cases a 40-fold increase as compared to the preimmune sera.

DISCUSSION

A polypeptide with the expected Mr for the predicted MS2-VP8' hybrid product was synthesized in E. coli at a level representing about 15% of the total cellular protein. This protein was found to form aggregates since, after lysis of the cells, it could be collected by low speed centrifugation. By this method, the MS2-VP8' polypeptide was enriched about threefold and was obtained at approximately 120 mg per litre of culture medium. Very frequently, recombinant polypeptides synthesized at this level are lethal for E. coli. However, by using a thermoinducible vector, the expression of the fused gene 4(5') could be very efficiently controlled, since no recognizable product was observed in the non-induced culture (see Fig. 3a, lane 1).

The viral amino acid sequence encoded in plasmid pSR4-2 lacked only the three amino-terminal amino acids of VP3 (S. López, unpublished results). With this additional information, we now know that the HhaI fragment cloned into the expression vector pPLc24 coded for amino acids 42 to 387 of VP3 (these amino acids correspond to amino acids n + 39 to n + 384 in López et al., 1985). Since the sequence of VP8 ends at amino acid 241 of VP3 (amino acid n + 238 in López et al., 1985), the hybrid polypeptide synthesized in E. coli should contain 85% of the carboxy-terminal sequence of VP8 and about 35% of the amino-terminal sequence of VP5. Overall, the viral sequences synthesized represent about 45% of VP3.

The presence of the MS2-VP8' polypeptide was detected in an immunoblot by antibodies raised against SA11 virions, indicating that some antigenic determinants of VP3 in the virion...
were preserved in the bacterial fusion product. This is supported by the fact that the hybrid polypeptide was able to induce antibodies that reacted with the native virus, as determined by haemagglutination inhibition and neutralization tests.

The MS2–VP8′ polypeptide seems to be a good immunogen since, when injected into mice, it was able to induce haemagglutination inhibiting antibodies at a titre similar to that obtained with the complete SA11 virion. However, the neutralizing antibody titre induced by the virus was about 20-fold higher than that induced by the chimeric product. A possible explanation is that antigenic determinants of any region of VP3 induce antibodies which inhibit viral haemagglutination, whereas those epitopes involved in neutralization may be more restricted such that only a subset of the antibodies raised against the bacterial fusion product would have neutralizing activity.

It is of note that the protein region in which the trypsin cleavage sites associated with the enhancement of rotavirus infectivity are located, is highly conserved among human rotaviruses belonging to different serotypes (López et al., 1986), suggesting that this portion of VP3 may be involved in the induction of heterotypic neutralizing antibodies. The capability of the MS2–VP8′ protein to elicit heterotypic neutralizing antibodies is currently under investigation.

The antigenically relevant portions of VP3 produced in bacteria, either alone or in combination with VP7 amino acid sequences containing neutralizing epitopes, may represent a means by which a safe, inexpensive and potentially effective vaccine against several different rotavirus serotypes may be produced.

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