Onderstepoort J. vet. Res. 52, 149–151 (1985)

THE USE OF RECOMBINANT DNA TECHNOLOGY FOR THE DEVELOPMENT OF A BLUETONGUE VIRUS SUBUNIT VACCINE

H. HUISMANS, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

HUISMANS, H., 1985. The use of recombinant DNA technology for the development of a bluetongue virus subunit vaccine. *Onderstepoort Journal of Veterinary Research*, 52, 149–151 (1985).

The double-stranded RNA gene coding for the surface antigen responsible for inducing neutralising antibodies has been isolated, converted to DNA, and cloned in the plasmid pBR322. So far, only plasmids containing inserts smaller than the gene have been obtained. Possible strategies for the development of a bluetongue virus subunit vaccine are discussed.

Bluetongue is one of the most important virus diseases of sheep in South Africa. It has been studied at the Veterinary Research Institute at Onderstepoort over a long period, dating back to the original discovery by Sir Arnold Theiler at the beginning of the century that the disease is caused by a virus. Thanks to this research we can today control the disease by means of a polyvalent attenuated live vaccine consisting of the 15 different serotypes of bluetongue virus (BTV) that are found in South Africa. A great deal of research on BTV, however, is still being carried out today and a large proportion of this research is directed towards solving some of the problems encountered with the vaccine. Most of these problems are associated with the polyvalent nature of the vaccine. Because of immunological interference, the immune response against a group of BTV serotypes is usually not as strong as against individually injected serotypes. Very important also is the problem of gene reassortment by means of which new recombinant viruses can be generated by the simultaneous infection with a number of closely related virus serotypes (Sugiyama, Bishop & Roy, 1981). These new viruses, which are mixtures of previously existing serotypes, increase the problems associated with the antigenic diversity in BTV.

At least a few of the problems can be solved by the use of subunit vaccines and the synthesis of such vaccines by means of the techniques developed in the field of biotechnology. The discussion of this particular approach in the case of BTV requires some understanding of the biochemistry of the virus and I will therefore briefly outline some of the more important aspects of this subject.

The history of BTV biochemistry dates back to the late sixties when Verwoerd purified the virus for the first time and was able to show that it contained double-stranded RNA (dsRNA) (Verwoerd, 1969). It was later shown that the RNA consists of 10 segments and that each segment contains the genetic information for the synthesis of one of the 10 virus-specified polypeptides (Verwoerd, Els, De Villiers & Huismans, 1972). Seven of these proteins have been identified as components of the double-layered protein coat of the virus. The outer capsid layer is composed of 2 proteins, P2 and P5. One of the major aims of the current biochemical research on BTV is to relate biological characteristics of BTV, such as pathogenicity, antigenic diversity, enzyme activities, etc., to specific functions of the viral proteins.

With respect to the problems associated with vaccination and antigenic diversity, it was important to determine which of the viral proteins are involved in the induction of neutralising antibodies. It was found that P2, a protein on the surface of the BTV particle, determines serotype specificity and induces neutralising antibodies (Huismans & Erasmus, 1981). This raised the possibility of using P2 as a single protein subunit vaccine. Similar observations in viruses, such as foot-and-mouth disease, influenza and rabies viruses, has stimulated quite extensive research over the past few years (Bachrach, 1982). Much of the research effort has been devoted to the production of single proteins by means of cloning or to the production of synthetic vaccines. In the case of BTV, we were fortunate in being able to produce large amounts of purified P2 by dissociation from purified virus (Huismans, Van der Walt, Cloete & Erasmus, 1983). The method does not require strong detergents, such as SDS, and the protein can be used for immunological studies. Injection of sheep with purified P2 results in the production of antibodies that precipitate P2 exclusively (Fig. 1.).

When the sheep are injected with 3 successive injection of 100 μg of purified P2, plaque reduction titres in the order of 128 are obtained. These sheep are fully protected against challenge with virulent virus. When sheep are injected with 1/10th of this amount, there are no detectable neutralising antibodies, but the sheep are nevertheless largely protected against infection with an homologous virulent serotype (Huismans, Van der Walt & Erasmus, 1984).

These results indicate that P2 could be used as a subunit vaccine. Unfortunately, the method for purifying P2 is not suitable for large scale vaccine production. An alternative is to develop production methods based on genetic manipulation techniques. Such an approach would require that the genetic material coding for the synthesis of P2 be combined with the DNA of a bacterium under such conditions that the bacterium expresses the information by synthesizing large amounts of P2. P2 is coded for by dsRNA segment 2. One of the first steps in the cloning strategy was therefore to isolate segment 2 and convert it to DNA. The method and the different steps used in this conversion are essentially those described by Cashdollar, Esparza, Hudson, Chimelo, Lee & Joklik, 1982. The virus-specific DNA was then inserted into the Pst 1 site of *Escherichia coli* plasmid pBR322 under such conditions that Pst 1 sites are generated at both ends of the insert. The recombinant plasmids were isolated by screening for specific antibiotic resistance markers and characterized by size, restriction enzymes and hybridization with a ^{32}P-labelled DNA probe made with BTV-mRNA as template.

Cloning was also carried out with unfractionated dsRNA as starting material . This so-called "shotgun" approach yielded a very large number of plasmids that contained BTV-specific inserts. Unfortunately, most of these inserts were very small. From a total of 3000 clones 700 were analysed and of these only about 20 had inserts of 1000 base pairs or more. Only 2 had inserts of more than 2000 base pairs. The electrophoretic separation of a few of these plasmids is shown in Fig. 2. Only one of these, p179, has an insert of just more than 2000 base pairs.

The largest plasmid obtained during cloning of the isolated P2-gene (p4) contained an insert of just more than a 1000 base pairs. The results obtained when the

FIG. 1 Autoradiogram demonstrating the electrophoretic separation of labelled BTV proteins (A), and the exclusive immune precipitation of P2 with a sheep serum prepared against purified P2 (B)

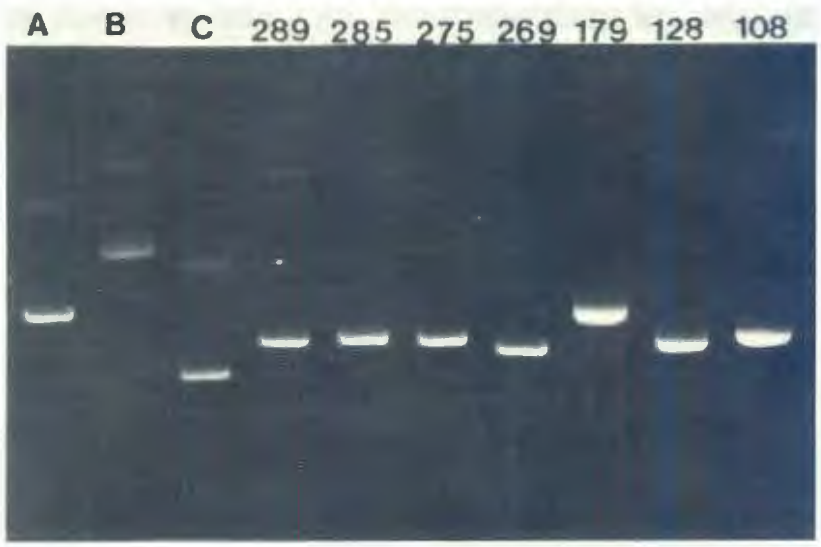

FIG. 2 Electrophoretic separation of a few of the plasmids with BTV-specific inserts obtained in the "shotgun" cloning experiment. The 1 % agarose gels were stained with ethidium bromide. Plasmid numbers are as indicated. Controls are pBR322 (C) and pBR322 with a 2500 base pair insert (A) and a 5000 base pair insert (B)

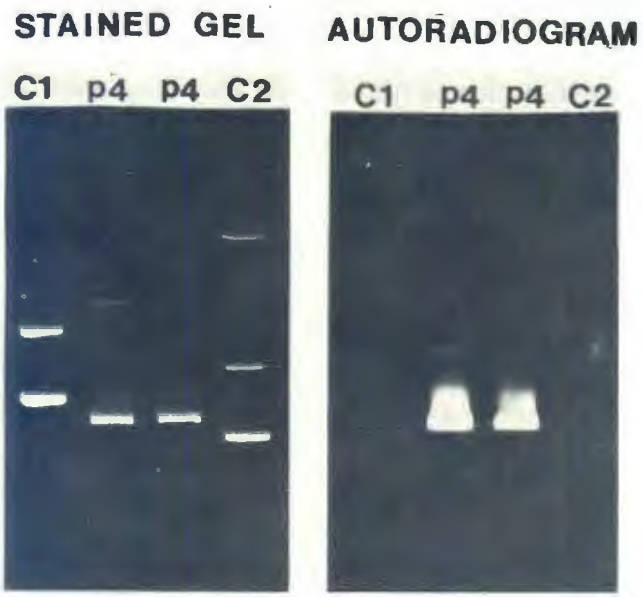

FIG. 3 Electrophoretic separation of plasmid p4 together with pBR322 (C2) and pBR322 with a 2500 base pair insert (C1). The same gel after hybridization with a ^{32}P-labelled BTV-specific probe and autoradiography is shown on the right

plasmid DNA was hybridized with a ^{32}P-labelled BTV specific mRNA probe are shown in Fig. 3. Hybridization was carried out in the gel according to the method of Purello & Balazs (1983) after electrophoretic separation of the plasmids on agarose gels. The autoradiogram on the right in Fig. 3 proves that plasmid p4 contains a BTV-specific insert.

Restriction enzyme analysis of 2 of the plasmids with BTV-specific inserts is shown in Fig. 4. When plasmid p4 is digested with Pst 1, an insert of approximately 1200 base pairs is excised (Fig. 4H). This insert represents about 1/3 of the total gene coding for P2.

When p179 was digested with Pst 1, three small DNA fragments were found (Fig. 4A), which indicates that the insert itself contains two Pst 1 cleavage sites. The combined size of the 3 fragments suggests that the insert has a total length of just more than 2000 base pairs. This was confirmed by incomplete digestion of p179 with Pst 1. A preliminary assignment of the p179 insert to genome segment 3 has been made.

The complete S2 gene coding for the neutralisation specific antigen of BTV has therefore not been cloned yet. Many of the plasmids with inserts of 1000 base pairs or more, however, may well contain full-sized cloned copies of some of the smaller BTV genes. By introducing a step during which the virus-specific DNA is sized before insertion into plasmid DNA, it may be possible to increase the average size of the inserts that are found. Such experiments have already been carried out.

The insertion of a piece of BTV-specific DNA into a plasmid does not mean, however, that this new information will necessarily be expressed. For expression the insert has to be under the control of a suitable promoter that will ensure transcription of the corresponding mRNA. This remains to be done in the case of BTV before any test for protein synthesis can be carried out.

Large scale synthesis of P2 in a bacterial host is not the only object of cloning, however. In the case of the foot-and-mouth disease virus, small synthetic peptides were shown to have significant potential as vaccines (Bittle, Houghton, Alexander, Shinnick, Sutcliffe, Lerner, Rowlands & Brown, 1982). These peptides are about 14–20 amino acids long and represent an immunologically important region on the much larger serotype- and neutralisation-specific antigen of the virus. Injection with such peptides not only provides a significant measure of protection against challenge with the whole virus but, in certain cases, the neutralising antibodies that are raised are less serotype specific, a very important consideration with respect to BTV. An investigation of this exciting possibility requires, however, that the sequence of the S2 gene of BTV be known. The developement o. sequencing techniques and their application over the last number of years have progressed at an amazing rate. To a large degree this has been brought about by the availability of large amounts of cloned DNA representing specific genes. Cloning of the gene that codes for P2 is

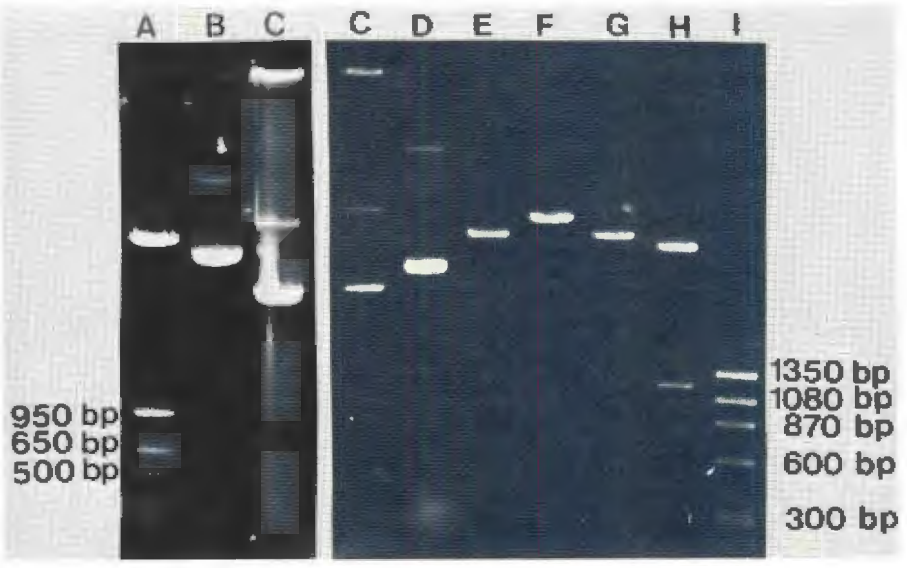

FIG. 4 Restriction enzyme analysis of plasmids p179 and p4; p179 Pst 1 digest (A); p179 (B); pBR322 (C); p4 (D); pBR322 BAM H1 digest (E); p4 BAM H1 digest (F); pBR322 Pst 1 digest (G); p4 Pst 1 digest (H); Phi X DNA-Hae III fragments as size markers (I). Separation was on 1% agarose gels and the gels were stained with ethidium bromide

therefore also a prerequisite first step in determining the sequence of the gene which will in turn allow us to derive the sequence of the corresponding P2 protein. This will enable us to investigate further the possibility of the synthesis and potential use of synthetic small polypeptides as vaccines.

REFERENCES

BACHRACH, H. L., 1982. Recombinant DNA technology for the preparation of subunit vaccines. *Journal of the American Veterinary Association*, 181, 992–999.

BITTLE, J. L., HOUGHTON, R. A., ALEXANDER, H., SHINNICK, T. M., SUTCLIFFE, J. G., LERNER, R. A., ROWLANDS, D. J. & BROWN, F., 1982. Protection against foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence. *Nature*, 298, 30–33.

CASHDOLLAR, L. W., ESPARZA, J., HUDSON, G. R., CHIMELO, R., LEE, P. W. L. & JOKLIK, W. K., 1982. Cloning of the double-stranded RNA genes of reovirus: Sequences of the cloned S2 gene. *Proceedings of the National Academy of Sciences* U.S.A., 79, 7644–7648.

HUISMANS, H. & ERASMUS, B. J., 1981. Identification of the serotype-specific and group-specific antigens of bluetongue virus. *Onderstepoort Journal of Veterinay Research*, 48,51–58.

HUISMANS, H., VAN DER WALT, N. T., CLOETE, M. & ERASMUS, B. J., 1983. The biochemical and immunological characterization of bluetongue virus capsid polypeptides. *In:* Double-stranded RNA viruses. Elsevier Science Publishing Co. Inc. p. 165–172.

HUISMANS, H., VAN DER WALT, N. T. & ERASMUS, B. J., 1985. Immune response against the purified serotype specific antigen of bluetongue virus and initial attempts to clone the gene that codes for the synthesis of this protein. *In:* Bluetongue and related arbiviruses. Alan R. Liss, Inc., New York, p. 347–353.

PURELLO, M. & BALAZS, I., 1983. Direct hybridization of labelled DNA to DNA in agarose gels. *Analytical Biochemistry*, 128, 393–397.

SUGIYAMA, K., BISHOP, D. H. L. & ROY, P., 1981. Analysis of the genomes of bluetongue viruses recovered in the United States. I. Oligonucleotide fingerprint studies that indicate the existence of naturally occurring reassortant BTV isolates. *Virology*, 114, 210–217.

VERWOERD, D. W., 1969. Purification and characterization of bluetongue virus. *Virology*, 38, 203–212.

VERWOERD, D. W., ELS, H. J., DE VILLIERS, E-M. & HUISMANS, H., 1972. Structure of the bluetongue virus capsid. *Journal of Virology*, 10, 783–794.