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(54) **PROTEINES D'ANTIGENE NUCLEOCAPSIDIQUE D'HEPADNAVIRUS CHIMERIQUE**

(54) **CHIMAERIC HEPADNAVIRUS CORE ANTIGEN PROTEINS**

(57) Particles, useful as a delivery system for an epitope, are composed of a chimaeric hepadnavirus core antigen protein wherein a foreign amino acid sequence comprising an epitope is inserted in or replaces all or part of the sequence of amino acid residues from 68 to 90 in the case where the core antigen is hepatitis B core antigen or the corresponding amino acid sequence in the case of the core antigen of another hepadnavirus.

ABSTRACT

Particles, useful as a delivery system for an epitope, are composed of a chimaeric hepadnavirus core antigen protein wherein a foreign amino acid sequence comprising an epitope is inserted in or replaces all or part of the sequence of amino acid residues from 68 to 90 in the case where the core antigen is hepatitis B core antigen or the corresponding amino acid sequence in the case of the core antigen of another hepadnavirus.

CHIMAERIC HEPADNAVIRUS CORE ANTIGEN PROTEINS

This invention relates to the construction of chimaeric hepadnavirus core antigen proteins.

Hepatitis B virus is a hepadnavirus virus with a partly double stranded genome of 3200 nucleotides. The viral DNA is surrounded by the viral coded core antigen (HBcAg) which is enclosed by the similarly coded surface antigen (Robinson, Ann. Rev. Microbiol. 31, 357-377, 1977). Removal of the surface antigen by mild detergent treatment leaves a core particle 27nm in diameter composed of HBcAg and the viral DNA. HBcAg has been expressed in microbial cells as the native polypeptide and as a derivative fused to the terminal eight residues of beta-galactosidase (see Murray et al, EMBO J. 3, 645-650, 1984 for refs).

When synthesized in E. coli the core protein self assembles into 27nm particles which can be visualized under the electron microscope (Cohen and Richmond, Nature, 296, 677-678, 1982) and which are immunogenic in laboratory animals (Stahl et al, Proc. Natl. Acad. Sci. USA 79, 1606-1610, 1982). The amino acid sequence of the core antigen shows a region towards the carboxy terminus which is homologous with that found in protamines (DNA binding proteins). By inference, it has been suggested that this part of the molecule interacts with DNA during assembly of core particles (Pasek et al, Nature, 282, 575-579, 1979).

The use of recombinant particles comprising

hepatitis B core antigen and heterologous protein sequences as potent immunogenic moieties is well documented. We have previously shown that addition of heterologous sequences to the amino terminus of the protein results in the spontaneous assembly of particulate structures on the surface of which the heterologous epitope is presented at high density and which are highly immunogenic when inoculated into experimental animals (Clarke et al, Nature 330, 381-384, 1987). Similar results have been reported by other groups using our system (e.g. Chang et al, 2nd International Symposium on positive strand RNA viruses, Vienna, Austria, 1989, abstract 010).

Subsequent experiments by other groups have shown that it is also possible to replace approximately 40 amino acids from the carboxy terminus of the protein with heterologous sequences and still maintain particle morphogenesis (Stahl & Murray, Proc. Natl. Acad. Sci. USA, 86 6283-6287, 1989). Moreover these particles are also immunogenic although, apparently, less so than the amino terminal fusions.

Despite the fact that these fusion particles induce excellent immune responses against the added epitope there still remains room for improvement from several points of view. Firstly, the immune responses against the added epitope, although excellent, do not compare with the parallel responses generated against the HBcAg sequences. Secondly, in both the amino and carboxy terminal fusions the

added epitope possesses inherent flexibility because it is only covalently bound at one end. This may be disadvantageous for conformationally rigid epitopes. Thirdly, a model of the structure of hepatitis B core antigen has predicted that, naturally, both the amino and carboxy termini of the protein are located internally within the particles (Argos & Fuller, EMBO J. 1, 819-824, 1988). It is only the inherently hydrophilic nature of most of the heterologous epitopes which directs them to the surface.

We have now located a region of high immunogenicity in a surface region of HBcAg particles. A vector encoding HBcAg and having a restriction site in this region of high immunogenicity has been constructed. Foreign sequences have been inserted at the restriction site, enabling chimaeric HBcAg proteins to be expressed. The chimaeric proteins self-assemble into particles having a foreign epitope exposed on their surface.

Accordingly, the present invention provides particles composed of a chimaeric hepadnavirus core protein wherein a foreign amino acid sequence comprising an epitope is inserted in or replaces all or part of the sequence of amino acid residues from 68 to 90 in the case where the core antigen is HBcAg or the corresponding amino acid sequence in the case of another hepadnavirus core antigen. HBcAg residues are numbered according to Ono et al, Nucl. Acid. Res. 11, 1747-1757, 1983. Corresponding residues of the core protein of another hepadnavirus may be determined by

lining up the sequences of HBcAg and the other core protein.

The chimaeric protein particles can be used to raise antibody specific for the epitope carried by the chimaeric protein. Antibody can therefore be raised in a mammal by administering to the mammal an effective amount of the particles composed of the chimaeric protein wherein the foreign sequence comprises an epitope capable of inducing antibody of the desired specificity. The chimaeric protein particles may be presented for this purpose as a component of a pharmaceutical or veterinary composition also comprising a pharmaceutically or veterinarily acceptable carrier.

The invention also provides a DNA sequence encoding a hepadnavirus core protein and having (a) a restriction site within the sequence encoding HBcAg amino acid residues 68 to 90 or the corresponding sequence of the core protein of another hepadnavirus or (b) two restriction sites flanking the sequence encoding HBcAg amino acid residues 68 to 90, a part of the sequence encoding HBcAg amino acid residues 68 to 90 or the corresponding sequence of the core protein of another hepadnavirus. Where HBcAg codons 68 to 90 or their counterpart codons for the core protein of another hapadnavirus have been deleted completely or in part, the restriction site is located appropriately in the sequence remaining. Where two restriction sites are provided, typically they are cut by the same restriction enzyme.

A vector can be constructed which incorporates such a DNA sequence. The vector can be provided in a host. The vector provides a starting point for the preparation of a vector capable of expressing the chimaeric protein of the invention. For this purpose, a DNA sequence needs to be constructed which encodes the chimaeric protein. More particularly, a vector is required which incorporates such a DNA sequence and which is capable, when provided in a suitable host, of expressing the chimaeric protein.

A vector capable of expressing the chimaeric protein is prepared by inserting a DNA sequence encoding the foreign sequence into a vector which encodes a hepadnavirus core protein and which has a restriction site or sites (a) or (b) as above. Preferably a restriction site (a) occurs at HBcAg codons 80 and 81 or at the corresponding codons for the core protein of another hepadnavirus. Alternatively, two restriction sites (b) may be provided at HBcAg codons 68 and 69 at one flank and at 80 and 81 at the other flank or, again, at the corresponding codons for the core protein of another hepadnavirus. The resulting vector encoding the chimaeric protein is typically provided in a compatible host.

To obtain the chimaeric protein, a host is cultured under such conditions that the chimaeric protein is expressed. The host is provided with an expression vector encoding the chimaeric protein. The chimaeric protein self-assembles into particles when expressed, and can then

be isolated. These particles closely resemble the 27 nm core particles composed of HBcAg and viral DNA which can be obtained by denaturing hepatitis B virus. The foreign epitope is exposed on the outer particle surface.

The chimaeric protein comprises a foreign amino acid sequence comprising an epitope. By "foreign" is meant that the sequence is not part of the sequence of the hepadnavirus core protein. The foreign sequence inserted into or replacing all or part of HBcAg amino acid residues 68 to 90 is not therefore part or all of the insert of 39 amino acids near the predicted position of the HBe1 epitope of avian hepatitis viruses, in particular of viruses from ducks (Feitelson and Miller, Proc. Natl. Acad. Sci. USA, 85, 6162-6166, 1988). The hepadnavirus core protein portion of the chimaeric protein is typically a mammalian hepadnavirus core antigen, in particular the human HBcAg or woodchuck WHcAg. Hepatitis B virus adw serotype HBcAg may be used.

Any foreign epitope, i.e. an epitope which is not an epitope of a hepadnavirus core protein, can be presented as part of the chimaeric protein. The epitope is a sequence of amino acid residues capable of raising antibody. The epitope may be an epitope capable of raising neutralising antibody, for example an epitope of an infectious agent or pathogen such as a virus or bacterium. It may be an epitope of a non-infectious agent such as a growth hormone. The foreign sequence may comprise repeats of an epitope, for example up to eight or up to four copies of an epitope. Two

copies of an epitope may therefore be present in the foreign sequence. A foreign sequence may comprise two or more different epitopes, for example three or four.

As examples of viruses whose epitopes may be presented there may be mentioned hepatitis A virus, hepatitis B virus, influenza virus, foot-and-mouth disease virus, poliovirus (PV), herpes simplex virus, rabies virus, feline leukaemia virus, human immunodeficiency virus type 1 (HIV-1), HIV-2, simian immunodeficiency virus (SIV), human rhinovirus (HRV), dengue virus and yellow fever virus. The epitope presented by the chimaeric protein may be therefore an epitope of HBsAg, of the pre-S region of HBsAg or of HRV2.

The foreign sequence in the chimaeric protein may be up to 100, for example up to 50, amino acid residues long. The foreign sequence may therefore be up to 40, up to 30, up to 20 or up to 10 amino acid residues in length. The foreign sequence comprises the epitope against which it is desired to induce antibody. The foreign sequence may also comprise further amino acid residues at either or both ends of the epitope.

Where further amino acid residues are present, these may be determined by the manipulations necessary to insert DNA encoding a desired foreign epitope into a vector encoding a hepadnavirus core antigen. They may be the amino acids which naturally flank the epitope. Up to 10, for example up to 4, further amino acids may be provided at

either or each end of the foreign epitope.

The foreign sequence may be inserted in the sequence of HBcAg residues from 68 to 90, for example 69 to 90, 71 to 90 or 75 to 85 or corresponding residues of another hepadnavirus core protein. Most preferred is to insert the foreign sequence between HBcAg amino acid residues 80 and 81 or corresponding residues of another hepadnavirus core protein. Alternatively, all or part of the sequence of core protein residues may be replaced by the foreign sequence. HBcAg amino acid residues 75 to 85, 80 and 81 or preferably 70 to 79 or corresponding residues of another hepadnavirus core protein may therefore be replaced by the foreign sequence. Where a foreign sequence replaces all or part of the native core protein sequence, the inserted foreign sequence is generally not shorter than the HBcAg sequence it replaces.

A second foreign amino acid sequence may be fused to the N-terminus or C-terminus of the amino acid sequence of the core protein. This second foreign sequence may also comprise an epitope. This epitope may be identical to or different from the epitope inserted into or replacing all or part of HBcAg amino acid residues 68 to 90 or the corresponding residues of the core protein of another hepadnavirus (the first epitope). Any foreign epitope may be present as the second epitope, as described above in connection with the first epitope. The length and construction of the foreign sequence containing the second

epitope may also be as described above in connection with the first epitope.

In order to prepare the chimaeric protein, an expression vector is first constructed. Thus a DNA sequence encoding the desired chimaeric protein is provided. An expression vector is prepared which incorporates the DNA sequence and which is capable of expressing the chimaeric protein when provided in a suitable host. Appropriate transcriptional and translational control elements are provided, including a promoter for the DNA sequence, a transcriptional terminal site, and translational start and stop codons. The DNA sequence is provided in the correct frame such as to enable expression of the polypeptide to occur in a host compatible with the vector.

An appropriate vector capable of expressing the chimaeric protein may be constructed from an HBcAg expression vector having a restriction site (a) or two restriction sites (b) as above. The restriction site (a) may be provided within the DNA sequence encoding HBcAg amino acid residues 71 to 90 or the counterpart residues of the core protein of another hepadnavirus, for example.

A DNA sequence encoding the foreign amino acid sequence is inserted into the HBcAg expression vector at the restriction site (a) or in place of the DNA sequence flanked by restriction sites (b). The HBcAg expression vector is digested with the appropriate restriction endonuclease(s) and dephosphorylated. The DNA sequence encoding the foreign

sequence is ligated into the cut expression vector. The inserted DNA sequence is typically prepared by standard techniques of oligonucleotide synthesis.

A or each restriction site in the HBcAg expression vector is preferably provided in the HBcAg coding sequence such that the HBcAg amino acid sequence is not altered. A restriction site (a) may occur at HBcAg codons 80 and 81 or the counterpart codons for another hepadnavirus core protein. Preferably, a NheI site is provided in the HBcAg coding sequence at codons 80 and 81. E. coli XL-1 Blue* harbouring plasmid pPV-Nhe, which is an HBcAg expression vector provided with such a NheI site, was deposited at the National Collection of Industrial and Marine Bacteria, Aberdeen, GB on 12 September 1989 under accession number NCIMB 40210.

An alternative or additional preferred restriction site spans HBcAg codons 68 and 69 or the counterpart codons for another hepadnavirus core protein. Suitably, a NheI site (underlined) is provided as follows:

	67	68	69
	ACG	CTA	GCT
	T	L	A

E. coli XL-1 Blue harbouring plasmid pPN2, which is an HBcAg expression vector provided with a NheI site at

* Trade-mark

codons 80 and 81 and with a NheI site at codons 68 and 69 as above, was deposited at the National Collection of Industrial and Marine Bacteria, Aberdeen, GB on 20 August 1990 under accession number NCIMB 40312.

Introduction of a novel restriction site can be achieved in either of two ways. First it may be achieved by replacement of a small restriction fragment coding for this region by a series of synthetic oligonucleotides coding for this region and incorporating the novel restriction site (see Example 1).

Secondly it may be achieved by site directed mutagenesis of the same coding region (see Example 5). This may typically be carried out by initially sub-cloning a restriction fragment representing this region into a vector such as M13mp18 which can produce single stranded DNA. Site directed mutagenesis may then be achieved using specific mismatched synthetic oligonucleotides by standard methods. Such a mutated restriction fragment can then be replaced into the parent gene in a suitable expression vector.

In the case of an HBcAg expression vector having all or part of the HBcAg coding sequence from amino acids 68 to 90, for example 71 to 90, replaced by a restriction site, a DNA sequence encoding the foreign amino acid sequence may be inserted as described above. This DNA sequence may encode, besides a Lys residue, one or more natural HBcAg residues so that part of the natural HBcAg amino acid sequence is provided between residues 68 and 90. HBcAg

residues 68, 69 and 70 may be provided in this way, for example.

The expression vectors encoding a chimaeric protein are provided in an appropriate host. The chimaeric protein is then expressed. Cells harbouring the vector are grown/cultured so as to enable expression to occur. The chimaeric protein that is expressed self-assembles into particles. The chimaeric particles may then be isolated.

Any appropriate host-vector system may be employed. The vector may be plasmid. In that event, a bacterial or yeast host may be used for example E. coli or S. cerevisiae. Alternatively, the vector may be a viral vector. This may be used to transfect cells of a mammalian cell line, such as Chinese hamster ovary (CHO) cells, in order to cause polypeptide expression.

The chimaeric protein may be used as a vaccine for a human or animal. It may be administered in any appropriate fashion. The choice of whether an oral route or a parenteral route such as sub-cutaneous, intravenous or intramuscular administration is adopted and of the dose depends upon the purpose of the vaccination and whether it is a human or mammal being vaccinated. Similar criteria govern the physiologically acceptable carrier or diluent employed in the vaccine preparation. Conventional formulations, carriers or diluents may be used. Typically, however, the fusion protein is administered in an amount of 1-1000 μg per dose, more preferably from 10-100 μg per dose,

by either the oral or the parenteral route.

The following Examples illustrate the invention.

In the accompanying drawings:

Figure 1 shows plasmid pBc404 is shown. B, E and P denote restriction sites for BamHI, EcoRI and PstI respectively; tac denotes the tac promoter; ori denotes the origin of replication; bla denotes β-lactamase and SD denotes the Shine-Dalgarno sequence.

Figure 2 shows the construction of plasmid pPV-Nhe.

Reference Example: Preparation of HBcAg provided at its N-terminus with a short extension comprising PV1 Mahoney VP1 residues 95 to 104

An expression plasmid pPV404 was prepared from the parent plasmid pBc404 shown in Figure 1. E. coli JM101 harbouring pBc404 was deposited at the National Collection of Industrial and Marine Bacteria, Aberdeen, GB on 9 February 1989 under accession number NCIMB 40111. Synthetic oligonucleotides representing amino acids 95 to 104 of VP1 from PV1 Mahoney were ligated into pBc404 using T4 ligase by standard procedures. This resulted in pPV404. The synthetic oligonucleotides, how they anneal together and the coding sequence of the N-terminal extension are as follows:

1. AATTCAGATAATCCAGCTAGTACTACCAACAAAGATAAG (39)

2. GATCCTTATCTTTGTTGGTAGTACTAGCTGGATTATCTG (39)

AATTCAG ATAATCCAGC TAGTACTACC AACAAAGATA AG

GTC TATTAGGTCG ATCATGATGG TTGTTTCTAT TCCTAGG

10 20 30 40

ATGAATTCAGATAATCCAGCTAGTACTACCAACAAAGATAAGGATCC.....CORE

5 M N S D N P A S T T N K D K D

LINKER POLIOVIRUS

Example 1: Preparation of plasmid pPV-Nhe

A series of peptides 20 amino acids in length were chemically synthesised. These peptides overlapped each other by 10 amino acids and together represented the whole amino acid sequence of the core protein from hepatitis B virus and serotype. Each of these peptides was used to coat microtitre plates in carbonate coating buffer and was used in an enzyme-linked immunosorbent assay (ELISA) analysis with sera from guinea pigs. The guinea pigs had been inoculated with bacterially-expressed hepatitis B core particles.

One of the peptides, corresponding to amino acids 71 to 90, gave an extremely powerful reaction. This indicated that core particles had elicited antibodies in vivo which reacted with the linear peptide sequence. The sequence corresponded roughly to the reported HBe1 epitope of hepatitis B core particles (Williams and LeBouvier, Bibilotheca Haematologica 42, 71-75, 1976). Our results

suggested that the insertion into or replacement of this immunodominant region of HBcAg by a foreign epitope may result in a chimaeric protein displaying enhanced immunogenicity with regard to the foreign epitope. We therefore undertook to insert foreign sequences into the adw core sequence.

The strategy which we followed is shown in Figure 2. The initial plasmid was plasmid pPV404. This plasmid expresses large amounts of chimaeric particles in bacteria which are highly immunogenic in animals. The strategy involved the introduction of a unique NheI restriction site at amino acid positions 80-81 in the core gene. This does not result in an amino acid change in the core protein. The nature of the mutation is shown below:

Amino acid	L	E	D	P	A	S	R	D	L
adw	TTG	GAA	GAT	CCA	GCA	TCC	AGG	GAT	CTA
adw-NheI	TTG	GAA	GAT	CCA	GCT AGC		AGG	GAT	CTA
					Nhe1				

Initially plasmid pPV404 was digested with restriction enzymes XbaI and AccIII resulting in two fragments of 3.96 kbp and 340 bp. These fragments were separated by electrophoresis on low melting point agarose, excised and the smaller fragment was then further digested with XhoII resulting in 3 fragments as shown in Figure 2.

Concomitantly two oligonucleotides were synthesised

with sequences as shown in Figure 2. These oligonucleotides were annealed and phosphorylated by standard procedures such that they represented a linker sequence with XhoII compatible "sticky ends" and an internal NheI site. These oligonucleotides were then ligated into the 340 bp fragment by standard procedures to replace the 19 bp natural XhoII fragment. This ligated material was then ligated back into the large 3.96 kbp fragment and transformed into E. coli strain XL-1 Blue by standard methods.

The design of this strategy did not exclude the possibility of the natural 19 bp XhoII fragment reinserting itself into the vector. To select for bacteria harbouring plasmids containing the new NheI site, a culture was prepared from all the recombinant clones generated during the transformation. This culture was then used to extract plasmid DNA representing the whole "library" of colonies. After caesium chloride purification this DNA was digested with NheI.

Only those recombinant plasmids carrying the new linker would have been digested in this way resulting in linearisation of this population. Linear DNA was therefore purified from the rest of the undigested plasmid molecules by agarose gel electrophoresis and, after religation, was transformed back into E. coli to generate a pool of NheI positive transformants. Individual clones were analysed by restriction mapping. Those clones which were confirmed to have an inserted NheI site were further characterised by DNA

sequencing. The restriction map of one of the resulting clones pPV-Nhe, which possessed the correct sequence, is shown in Figure 2.

As previously stated, the design of the experiment ensured the maintenance of the correct HBcAg amino acid sequence. It was not surprising therefore that expression analysis after induction with isopropyl-beta-D-thiogalactopyranoside (IPTG) confirmed the presence of a high yield of PV-HBcAg particles.

Example 2: Preparation of particles composed of chimaeric HBcAg proteins

The ability of pPV-Nhe to express heterologous sequences was initially assessed by insertion of epitopes from human rhinovirus type 2 (HRV2) and hepatitis B surface antigen (HBsAg). This was achieved by insertion of synthetic oligonucleotides coding for each sequence flanked by NheI cohesive ends into NheI digested and dephosphorylated pPV-Nhe. The sequences inserted at the NheI site of pPV-Nhe are shown below:

HRV2 VP2

Epitope

A S V K A E T R L N P D L Q P T E C A S

CTAGCGTTAAAGCGGAAACGCGTTTGAACCCAGATCTGCAACCGACCGAATGCG

GCAATTTCGCCTTTGCGCAAACTTGGGTCTAGACGTTGGCTGGCTTACGCGATC

HBsAg 139-147

Epitope

A S G A C T K P T D G N C A G A S

CTAGCGGTGCATGCACAAAACCTACTGATGGTAACTGCGCAGGTG

GCCACGTACGTGTTTTGGATGACTACCATTGACGCGTCCACGATC

Plasmids ligated with the synthetic oligonucleotides were transformed into E. coli strain XL-1 Blue. Each new construct was designed so that a diagnostic internal restriction site was present allowing rapid screening of the resulting clones. The internal restriction sites were MluI for HRV2 and SphI for HBsAg. Resulting clones possessing correct restriction sites were cultured to high density in nutrient broth and expression of chimaeric proteins was induced by addition of IPTG to the medium. Following incubation for 6 to 8 hours at 37°C bacterial cells were harvested by centrifugation, lysed by standard procedures and expressed proteins analysed by PAGE, Western blotting and ELISA. The presence of particulate structures was determined by sucrose density gradient centrifugation.

Expression of chimaeric proteins comprising either the HRV2 epitope or the HBsAg was observed. The chimaeric proteins self-assembled into particles. Detailed expression analysis on the HRV2 epitope construct showed that expression levels were very high in bacteria, that particle formation was maintained and that the chimaeric protein reacted with anti-HRV2 sera by Western blotting. ELISA analysis also showed that the HRV2 epitope was exposed on the particle surface.

EXAMPLE 3: Preparation of further particulate chimaeric HBcAG proteins

Further epitopes have been inserted into pPV-Nhe. Synthetic oligonucleotides were ligated together to prepare a DNA fragment encoding the epitope and having cohesive Nhe I ends. The DNA fragment was inserted into Nhe I - digested and dephosphorylated pPV-Nhe. Plasmids ligated with the DNA fragment were transformed into

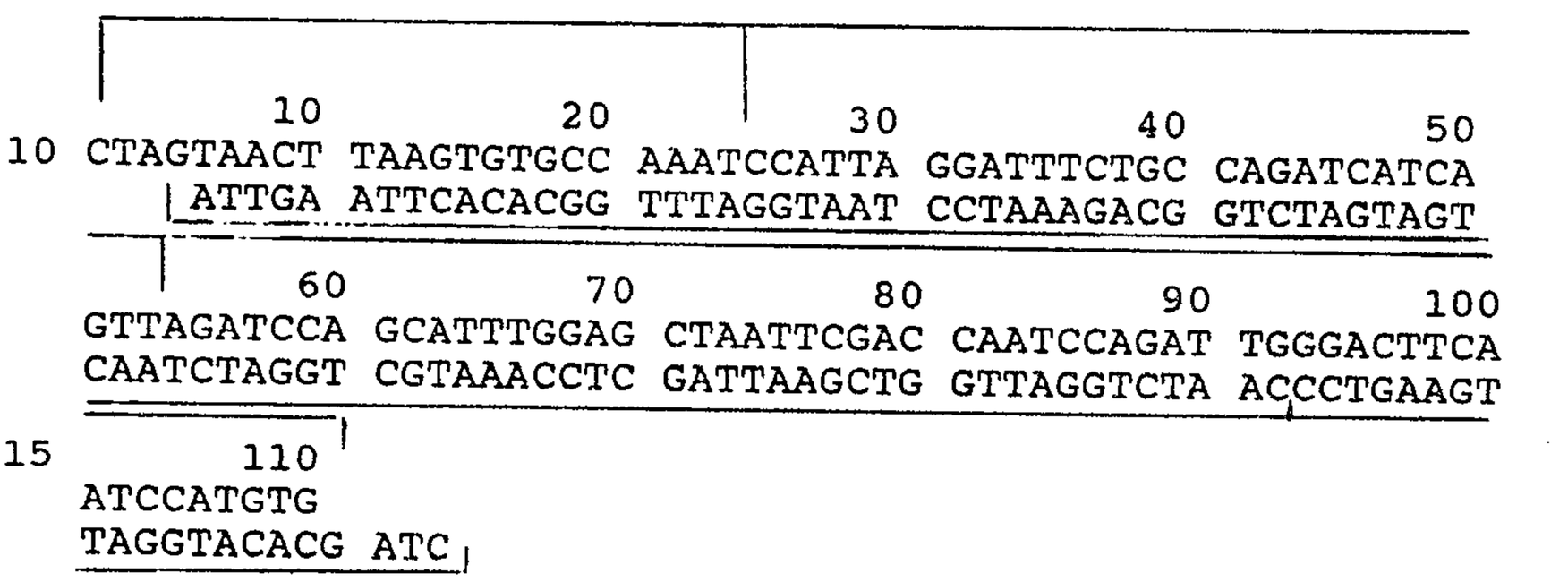
E. coli strain XL-1 Blue.

Clones were cultured to high density in nutrient broth and expression of chimaeric protein was induced by addition of IPTG to the medium. Following incubation for 6 to 8 hours at 37°C bacterial cells were harvested by centrifugation, lysed by standard procedures and expressed proteins analysed by PAGE, Western blotting and/or ELISA. The presence of particulate structures was determined by sucrose density gradient centrifugation.

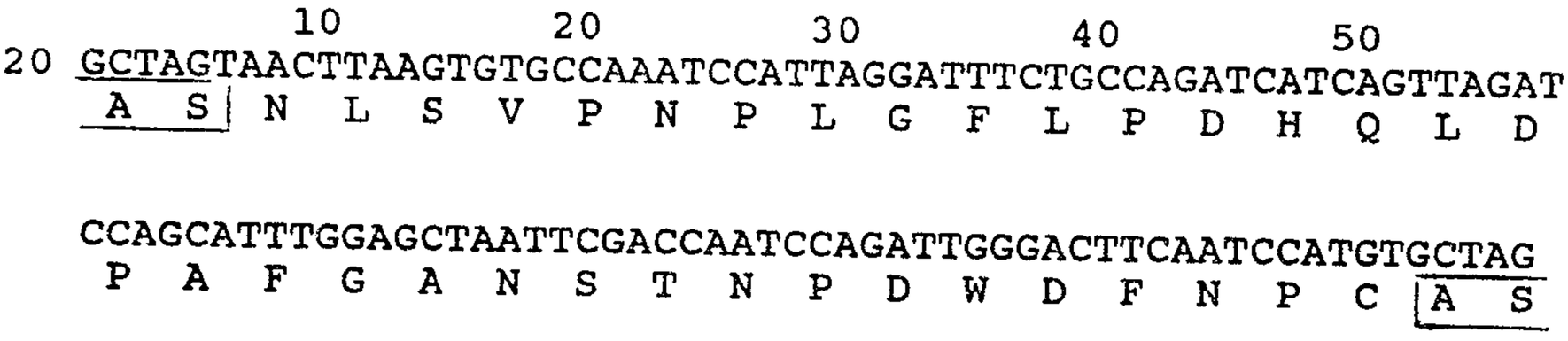
Chimaeric proteins presenting the following epitopes were obtained in this way. In each case the epitope was flanked by A-S residues due to the cloning procedure.

1. QE1-amino acid residues 15 to 47 from the Pre-S1 region of hepatitis B virus (HBV). This region is implicated in virus-cell interactions. The sequence was derived from the adw serotype.

(a) Synthetic oligonucleotides



(b) Coding sequence



2. QM2-133-144 from Pre-S2 region of HBV. This region is a sequential epitope in HBV and can protect chimpanzees from infection. The sequence was derived from the adw serotype.

(a) Synthetic oligonucleotides

```
          10          20          30          40
CTAGTGATC CGCGCGTGCG CGGCTTATAC TTACCGGCGG GAG
    ACTAG GCGCGCACGC GCCGAATATG AATGGCCGCC CTCGATC
```

(b) Coding sequence

```
          10          20          30          40
GCTAGTGATCCGCGCGTGCGCGGCTTATACTTACCGGCGGGAGCTAGC
 A   S  D   P   R   V   R   G   L   Y   L   P   A   G  A   S
```

3. QE2-120-153 from Pre-S2 region of HBV. This is a larger version of 2. The sequence was derived from the adw serotype.

(a) Synthetic oligonucleotides

```
          10          20          30          40          50
CTAGCATGC AATGGAATAG CACCGCGTTA CACCAAGCTT TGCAGGACCC
    GTACG TTACCTTACT GTGGCGCAAT GTGGTTCGAA ACGTCCTGGG

          60          70          80          90          100
TCGAGTACGT GGCTTATACT TACCGGCGGG AGGATCAAGC AGCGGCACCG
AGCTCATGCA CCGAATATGA ATGGCCGCCC TCCTAGTTCG TCGCCGTGGC

          110
TTAATCCGG
AATTAGGCCG ATC
```

(b) Coding sequence

```
          10          20          30          40          50
GCTAGCATGCAATGGAATAGCACCGCGTTACACCAAGCTTTGCAGGACCCTCGAGT
 A   S  M   Q   W   N   S   T   A   L   H   Q   A   L   Q   D   P   R   V

  60          70          80          90          100
ACGTGGCTTATACTTACCGGCGGGAGGATCAAGCAGCGGCACCGTTAATCC
  R   G   L   Y   L   P   A   G   G   S   S   S   G   T   V   N   P

110
GGCTAGC
A   S
```

4. pPD1-110-148, a couplex epitope from HBsAg.

(a) Synthetic oligonucleotides

```
        10         20         30         40         50
CTAGTATTC CTGGGTCAAC GACCACGAGC ACCGGACCAT GCAAGACGTG
   ATAAG GACCCAGTTG CTGGTGCTCG TGGCCTGGTA CGTTCTGCAC

          60         70         80         90        100
TACTACACCA GCACAAGGTA ACTCCAAGTT CCCGAGCTGC TGCTGCACAA
ATGATGTGGT CGTGTTCCAT TGAGGTTCAA GGGCTCGACG ACGACGTGTT

        110        120
AACCTACTGA TGGTAACTGC ACTG
TTGGATGACT ACCATTGACG TGACGATC
```

(b) Coding sequence

```
          10         20         30         40         50
GCTAGTATTCCTGGGTCAACGACCACGAGCACCGGACCATGCAAGACGTGTACTA
 A   S  I   P   G   S   T   T   T   S   T   G   P   C   K   T   C   T

    60         70         80         90        100        110
CACCAGCACAAGGTAACTCCAAGTTCCCGAGCTGCTGCTGCACAAAACCTACTGA
T   P   A   Q   G   N   S   K   F   P   S   C   C   C   T   K   P   T   D

          120
TGGTAACTGCACTGCTAGC
  G   N   C   T  A   S
```

5. pPA1-VP1 101-110 HAV (Hepatitis A virus)

(a) Synthetic oligonucleotides

```
                  471
CTAGCAATTCGAATAACAAGGAGTATACATTTCCGG
    GTTAAGCTTATTGTTCCTCATATGTAAAGGCCGATC
                  472
```

(b) Coding sequence

```
          10         20         30         40
GCTAGCAATTCGAATAACAAGGAGTATACATTTCCGGCTAGC
 A   S  N   S   N   N   K   E   Y   T   F   P  A   S
```

6. pPA2-VP1 13-24 HAV

(a) Synthetic oligonucleotides

```
                    473
  CTAGCACTGAACAGAATGTTCCGGATCCTCAGGTTGGAG
5      GTGACTTGTCTTACAAGGCCTAGGAGTCCAACCTCGATC
                    474
```

(b) Coding sequence

```
           10        20        30        40
  GCTAGCACTGAACAGAATGTTCCGGATCCTCAGGTTGGAGCTAGC
10 A  S  T  E  Q  N  V  P  D  P  Q  V  G  A  S
```

7. pPA3-VP3 61-83 HAV

(a) Synthetic oligonucleotides

```
  475                 466                 476
  CTAGCGCAGCACAATTTCCCTTCAATGCAAGCGATTCAGTCGGACAACAGATAAAG
15     GCGTCGTGTTAAAGGGAAGTTACGTTCGCTAAGTCAGCCTGTTGTCTATTTC
       478                 469                 477
  GTTATACCTGTGGATCCTG
  CAATATGGACACCTAGGACGATC
```

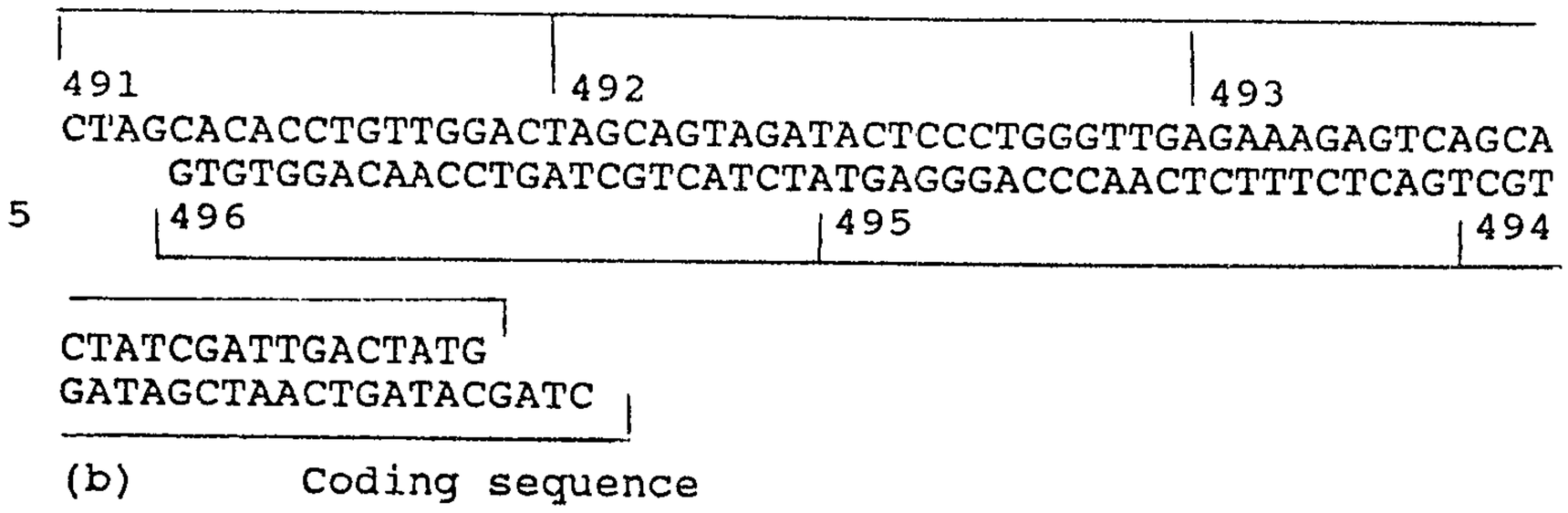
(b) Coding sequence

```
20         10        20        30        40        50
  GCTAGCGCAGCACAATTTCCCTTCAATGCAAGCGATTCAGTCGGACAACAGATAAA
   A  S  A  A  Q  F  P  F  N  A  S  D  S  V  G  Q  Q  I  K

     60        70        80
  GGTTATACCTGTGGATCCTGCTAGC
25   V  I  P  V  D  P  A  S
```

8. pPA4-VP1 160-182 HAV

(a) Synthetic oligonucleotides



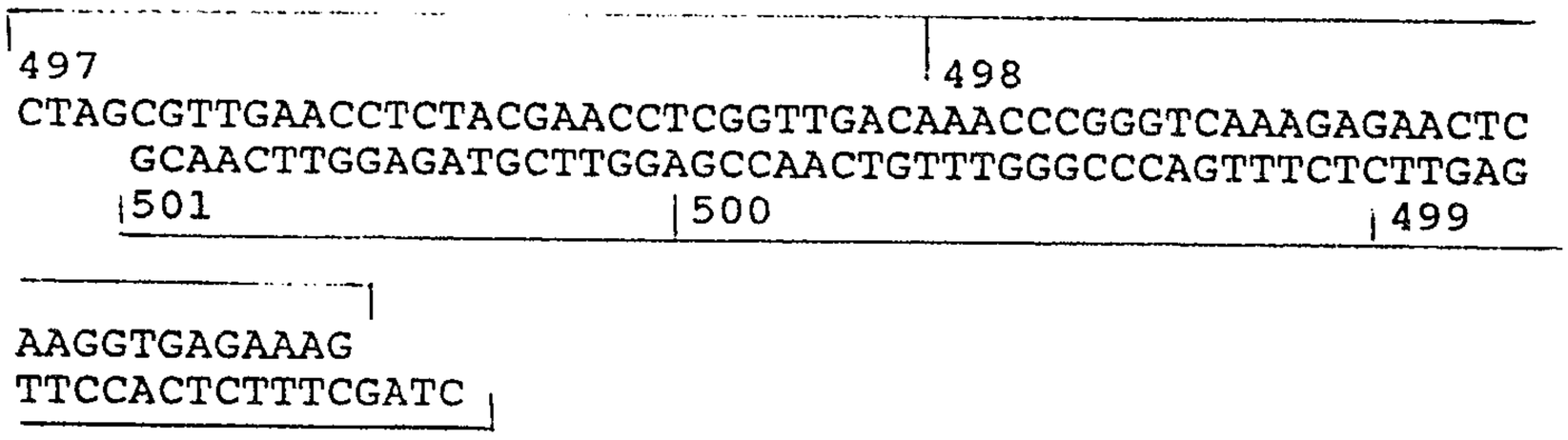
(b) Coding sequence

```
          10        20        30        40        50
GCTAGCACACCTGTTGGACTAGCAGTAGATACTCCCTGGGTTGAGAAAGAGTCA
 A  S  T  P  V  G  L  A  V  D  T  P  W  V  E  K  E  S

     60        70
GCACTATCGATTGACTATGCTAGC
 A  L  S  I  D  Y  A  S
```

9. pPA5-VP2 40-60 HAV

(a) Synthetic oligonucleotides



(b) Coding sequence

```
          10        20        30        40        50
GCTAGCGTTGAACCTCTACGAACCTCGGTTGACAAACCCGGGTCAAAGAGAAC
 A  S  V  E  P  L  R  T  S  V  D  K  P  G  S  K  R  T

     60        70
TCAAGGTGAGAAAGCTAGC
 Q  G  E  K  A  S
```

10. Amino acids 735-752 from gp41 of HIV-1. This is a potential neutralising epitope for HIV.

(a) Synthetic oligonucleotides

```
         10          20          30          40          50
5 CTAGCGACC GCCCTGAGGG CATCGAGGAA GAGGGCGGTG AGCGCGATCG
    GCTGG CGGGACTCCC GTAGCTCCTT CTCCCGCCAC TCGCGCTAGC
          60
    TGATCGTTCAG
    ACTAGCAAGTCGATC
```

10 (b) Coding sequence

```
          10          20          30          40          50
    GCTAGCGACCGCCCTGAGGGCATCGAGGAAGAGGGCGGTGAGCGCGATCGTGAT
    A  S  D  R  P  E  G  I  E  E  E  G  G  E  R  D  R  D

        60
15  CGTTCAGCTAGC
     R  S  A  S
```

11. Epitope from feline leukaemia virus gp70 (197-219) implicated in induction of neutralizing antibodies.

a) Synthetic oligonucleotides

```
20        10          20          30          40          50
    CTAGTACTA TCACTCCACC ACAGGCCATG GGTCCAAACT TAGTCTTACC
        ATGAT AGTGAGGTGG TGTCCGGTAC CCAGGTTTGA ATCAGAATGG
```

```
          60          70          80
AGATCAAAAG CCACCAAGTC GTCAAG
TGTAGTTTTC GGTGGTTCAG CAGTTCGATC
```

b) Coding sequence

```
          10          20          30          40
GCTAGTACTATCACTCCACCACAGGCCATGGGTCCAAACTTA
 A  S  T  I  T  P  P  Q  A  M  G  P  N  L

          50          60          70          80
GTCTTACCAGATCAAAAGCCACCAAGTCGTCAAGCTAGC
 V  L  P  D  Q  K  P  P  S  R  Q  A  S
```

Example 4: Preparation of chimaeric protein having a HRV2 epitope at both the amino terminus and inserted between HBcAg residues 80 and 81

Oligonucleotides coding for the HRV2 VP2 epitope shown in Example 2 were inserted into the pPV-Nhe vector as specified in that Example. The recombinant vector was digested with EcoRI and BamHI. A band of approximately 4.4kb was purified by low melting point agarose gel electrophoresis. Synthetic oligonucleotides representing amino acids 156 to 170 of VP2 from HRV2 were ligated into the recombinant vector using T4 ligase by standard procedures. The synthetic oligonucleotides, how they anneal together and the coding sequence of the N-terminal extension were as follows:

1. AATTCAGTTAAAGCGGAAACGCGTTTG

2. AACCCAGATCTGCAACCGACCGAATGCCGG

3. GATCCCGGCATTCGGTCGGTTGCA

4. GATCTGGGTTCAAACGCGTTTCCGCTTTAACTG

5 AATTCAGTTAAAGCGGAAACGCGTTTGAACCCAGATCTGCAACCGACCGAATGCCGG
GTCAATTTCGCCTTTGCGCAAACTTGGGTCTAGACGTTGGCTGGCTTACGGCCTAG

```
                                                    30
ATG AAT TCA GTT AAA GCG GAA ACG CGT TTG AAC CCA GAT CTG CAA
 M   N   S   V   K   A   E   T   R   L   N   P   D   L   Q
  LINKER                                    HRV2
                    60
CCG ACC GAA TGC CGG GAT CC
 P   T   E   C   R   D
```

The resulting plasmid was transformed into E. coli strain XL-1 Blue. Clones were cultured to high density in nutrient broth. Expression of chimaeric protein was achieved as described in Example 2. Expressed proteins were analysed by PAGE, Western blotting and ELISA. The presence of particulate structures was determined by sucrose density gradient centrifugation.

Example 5: Preparation of plasmid pPN2 which enables replacement of part of HBcAg amino acid sequence

The entire HBcAg gene was subcloned into a vector capable of producing single stranded DNA. This was carried out specifically using a technique known as sticky foot mutagenesis. Initially the core gene from pPV-NheI (Example 1) was amplified by polymerase chain reaction using two oligonucleotides as shown below:

1) TACGCAAACCGGCTCTCCCCGAATTCGTTGACAATTAATCATCGGCT

lacZ tac

2) TTGGGAAGGGCGATCGGTGCGGATCCTAACATTCGAGATTCGCGAGA

lacZ HBcAg

The resulting fragment (600 base pairs) therefore has lacZ complementary sequences at each end. In parallel, single stranded uracil rich DNA was prepared from a commercial vector pBS-SK(+) (Stratagene) which contains a complete lacZ gene. This single stranded DNA was mixed with the PCR fragment whereon the newly introduced lacZ flanking regions annealed with the single stranded vector. This annealed duplex was then double stranded using DNA polymerase and trasfected into E. coli strain XL1-Blue. Colonies were assayed for presence of correct recombinant plasmid by restriction mapping.

This plasmid (Q9) therefore carries an entire copy of the PV-NheI HBcAg gene under transcriptional control of the lac promoter. It also, being pBS derived, is capable of producing a single stranded DNA. Such single stranded DNA (uracil rich) was therefore prepared and used to produce an additional NheI restriction site at amino acids 68 and 69 of the HBcAg protein. This was carried out by annealing a synthetic oligonucleotide with single stranded DNA from Q9, polymerising and removing parental template as before. The oligonucleotide used for the mutagenesis was:

GGAATTGATGACGCTAGCTACCTGGGTGGG

NheI

Recombinant DNA was transfected into E. coli XL-1 Blue and colonies analysed by direct sequencing of the resultant DNA by primer extension. The sequence of the mutated region in pPN2 is as shown with both NheI sites underlined. The region between the two restriction sites can be substituted with synthetic oligonucleotides coding for the required epitope.

ACG CTA GCT ACC TGG GTG GGT AAT AAT TTG GAA GAT CCA GCT AGC

T L A T W V G N N L E D P A S

Example 6: Animal tests

Immunisation protocol

Female Dunkin Hartley guinea-pigs weighing about 400g were each inoculated intramuscularly with a 0.5 ml dose of a chimaeric HBcAg protein preparation formulated in incomplete Freund's adjuvant (IFA). Groups of four animals were inoculated with a specified dose of purified core particles and boosted once at either 56 or 70 days with the same initial dose. Blood samples were taken at 14 day intervals throughout the experiment.

ELISA

Antipeptide, antivirus and anti-particle activity

in serum samples was measured by a modification of an indirect or double antibody sandwich ELISA method. In the sandwich ELISA polyclonal antipeptide serum (1:200) was used to capture serial dilutions of particles in PBS and 2% dried milk powder. In the indirect ELISA 2 μg/ml of peptide, particle or virus were coated directly onto microtitre plates. In each case the plates were washed and incubated with test serum samples. Following incubation at 37C for 1-2 hours plates were rewashed and anti-IgG-peroxidase conjugate was added. After a further hour at 37°C the plates were washed and an enzyme substrate (0.04% o-phenylenediamine and 0.004% hydrogen peroxide in 0.1M phosphate 0.05M citrate buffer) was added. The resulting colour development was stopped with sulphuric acid and the A492 was measured in a Titertek Multiskan (Flow Labs).

The A492 values obtained from dilutions of post-inoculation samples were plotted against the log10 reciprocal antiserum dilution and the antibody titre was calculated by reference to a negative standard (a 1:10 dilution of pre-inoculation serum).

Results

1. Guinea pigs were inoculated intramuscularly with 20 μg or 2 μg of particles obtained in Example 3.1 composed of the PreS1-HBcAg chimaeric protein. Bleeds were taken at regular intervals. In each case a peptide composed of the PreS1 insert in the chimaeric HBcAg protein (392), HBcAg with no

insert (control) and PreS1-HBcAg particles were coated onto enzyme-linked immunosorbent assay (ELISA) dishes and then assayed against dilutions of the antisera collected. The results are shown in Table 1. Very high levels of antipeptide, antiHBcAg and antiPreS1-HBcAg particles antibody were achieved.

2. The procedure was the same as in 1 except:

- guinea pigs were inoculated with particles obtained in Example 3.2 composed of the small PreS2 epitope-HBcAg chimaeric protein;

- a peptide composed of the PreS2 insert (393), HBcAg, PreS2-HBcAg particles and yeast-derived HBsAg particles with PreS2 epitopes incorporated were coated onto ELISA dishes.

The results are shown in Table 2. Very high levels of antibody were again induced in the guinea pigs.

3. The immune responses induced in guinea pigs and rabbits by particles composed of a chimaeric HBcAg protein in which a HRV2 VP2 epitope is inserted in accordance with the invention ("insert", Example 2) and by particles composed of a chimaeric HBcAg protein in which the same HRV2 VP2 epitope is fused to the amino terminus of HBcAg ("terminal") were compared. The "terminal" chimaeric HBcAg protein was prepared in accordance with the procedure described in JP-A-196299/88. The results are shown in Tables 3 and 4 and are ELISA endpoint titres (log_{10}). The ELISA plates were coated with either a peptide composed of the HRV2 VP2 epitope or HBV. The "insert" chimaeric HBcAg protein gave superior

results: higher anti-HRV peptide titres and lower anti-HBV titres.

4. The neutralising antibody responses were looked at of the guinea pigs and rabbits of 3. above. In particular, the responses of the animals to two inoculations of the "insert" particles were assayed. The results are shown in Table 5.

5. Rabbits and guinea pigs were inoculated intramuscularly with particles composed of the chimaeric HBsAg 139-147 epitope-HBcAg protein of Example 2. Anti-peptide 139-147 (α pep 448) and anti-HBsAg (αHBsAg) responses were determined using three doses of particles. The results are shown in Table 6. The data shown are titres (log_{10}) prior to a first booster inoculation at 42 days and at the final bleeds day 98. In terms of anti-HBsAg activity, good antibody levels were observed in final bleeds in rabbits and, particularly, in guinea pigs.

TABLE 1

		392 (2μg/ml)	HBcAg (2μg/ml)	PreS1-HBcAg (2μg/ml)
Group 1				
5 20μg	14	3.57	2.89	3.78
	28	4.48	3.73	4.28
	42	4.54	4.16	4.59
	71*	4.63	3.87	3.79
	77	5.39	4.20	4.44
10	84	4.69	4.24	4.46
	98*	5.15	4.32	4.73
Group 2				
2μg	14	2.73	3.15	3.30
	28*	4.20	3.69	3.57
15	42	4.22	3.39	3.39
	71*	4.16	3.77	3.93
	77	4.02	3.77	3.94
	84	4.15	3.95	3.90
	98*	4.60	4.41	4.31

20 ELISA end point titres $\log_{10}$

* mean of group of individuals

Table 2: Response of guinea pigs to preS2 (QM2/PE3) cores (small epitope)

(a) 20µg dose

Days post primary inoculation	Test antigen peptide 393	HBcAg	QM2 cores	HBsAg + preS2
0**	<1*	<1	<1	<1
14	2.5	2.2	3.3	2.1
28	3.2	3.0	4.1	3.1
42	3.6	3.3	4.2	3.4
70**	3.6	4.4	4.5	3.8
77	3.9	4.4	4.5	3.9
84	4.2	4.7	5.2	4.4
98	4.1	4.6	4.9	4.1

(b) 2µg dose

Drugs post primary inoculation		Test antigen			
	peptide 393	HBcAg	QM2 cores	HBsAg +preS2
5 0**	<1*	<1	<1	<1
14	2.0	2.0	3.0	1.9
28	2.1	2.6	3.5	2.2
42	2.3	3.3	3.3	2.3
70**	2.5	3.7	3.7	3.0
10 77	3.2	4.2	4.1	3.5
84	3.7	4.8	4.7	4.0
98	3.4	4.6	4.9	4.2

* $\log_{10}$ end point titre

** inoculations

Table 3: Comparative immunogenicity of "insert" and "terminal" HBcAg/HRV peptide fusion protein particles in guinea pigs

(a) Anti-HRV peptide

Peptide dose (µg)	28 days post primary		56 days post primary		28 days post boost	
	insert	terminal	insert	terminal	insert	terminal
1.5	3.6*	2.7	4.1	2.9	4.8	3.6
0.15	2.5	1.3	3.1	2.3	4.3	2.9
0.015	1.9	<1.0	2.1	<1.0	3.5	<1.0

(b) Anti-HRV

Peptide dose (µg)	28 days post primary		56 days post primary		28 days post boost	
	insert	terminal	insert	terminal	insert	terminal
1.5	3.9*	1.6	4.2	1.5	5.1	2.8
0.15	2.3	1.2	2.5	1.6	4.2	2.5
0.015	1.6	1.2	1.8	1.0	3.5	1.0

* ELISA endpoint titre (log_{10})

Table 4: Comparative immunogenicity of "insert" and "terminal" HBcAg/HRV peptide fusion protein particles in rabbits

(a) Anti-HRV peptide

Peptide dose (μg)	28 days post primary		56 days post primary		28 days post boost	
	insert	terminal	insert	terminal	insert	terminal
1.5	3.0*	2.2	3.2	2.4	4.2	2.9
0.15	2.7	1.5	2.4	1.5	3.1	1.7
0.015	2.1	N.D.	1.8	N.D.	2.7	N.D.

(b) Anti- HRV

Peptide dose (μg)	28 days post primary		56 days post primary		28 days post boost	
	insert	terminal	insert	terminal	insert	terminal
1.5	2.1*	1.2	2.5	1.1	4.0	1.4
0.15	1.8	1.1	2.0	1.1	2.8	1.5
0.015	1.7	N.D.	1.6	N.D.	2.4	N.D.

N.D. = not determined

* ELISA endpoint titre (log_{10})

TABLE 5

(a) Guinea pigs peptide dose (μg)	Neutralization titre 90% Plaque reduction
1.5	90, 30, 50, 25
0.15	20, 5
0.015	<5, <5

(b) Rabbits peptide dose (μg)	Neutralization titre 90% Plaque reduction
1.5	300, 5
0.15	10, 15
0.015	<5, <5

TABLE 6

				Ist Inoc.	2nd Inoc.
chimaeric HBsAg 139-147 HBcAg protein	Rabbits	20μg	αpep448	3.3	3.4
			αHBsAg	1.6	2.7
		2μg	αpep448	2.6	3.0
			αHBsAg	1.3	2.4
		0.2μg	αpep448	2.7	3.2
			αHBsAg	1.2	1.4
	Guinea Pigs	20μg	αpep448	3.9	5.1
			αHBsAg	3.6	5.0
		2μg	αpep448	3.0	4.3
			αHBsAg	1.9	4.0
		0.2μg	αpep448	2.1	4.0
			αHBsAg	<1	2.4

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Particles composed of a chimaeric hepadnavirus core antigen protein which includes a foreign amino acid sequence comprising an epitope, said foreign amino acid sequence being inserted in or replacing all or part of an amino acid sequence NLEDPASRDLV from residue 75 to residue 85 when the core antigen protein is hepatitis B core antigen protein or a corresponding amino acid sequence for a core antigen protein of another hepadnavirus.

2. Particles according to claim 1, wherein the foreign amino acid sequence is up to 40 amino acid residues in length.

3. Particles according to claim 2, wherein the foreign amino acid sequence is up to 20 amino acid residues in length.

4. Particles according to claim 1, 2 or 3, wherein the foreign amino acid sequence is inserted between hepatitis B core antigen protein residues AS corresponding to residues 80 and 81 or between corresponding residues of a core antigen protein of another hepadnavirus.

5. Particles according to claim 1, 2, 3 or 4, wherein the epitope is the epitope of a pathogen.

6. Particles according to claim 5, wherein the epitope is an epitope of hepatitis A virus, hepatitis B virus, influenza virus, foot-and-mouth disease virus,

poliovirus, herpes simplex virus, rabies virus, feline leukaemia virus, human immunodeficiency virus type 1 or 2, simian immunodeficiency virus, human rhinovirus, dengue virus or yellow fever virus.

7. Particles according to claim 1, 2, 3, 4, 5 or 6, wherein a second said foreign amino acid sequence is fused to the N-terminal of the amino acid sequence of the core protein.

8. A vector which comprises a DNA sequence encoding a chimaeric hepadnavirus core antigen protein including a foreign amino acid sequence comprising an epitope, which vector is capable of expressing the chimaeric protein when provided in a suitable host, the foreign amino acid sequence being inserted in or replacing all or part of an amino acid sequence NLEDPASRDLV from residue 75 to residue 85 when the core antigen protein is hepatitis B core antigen protein or a corresponding amino acid sequence for a core antigen protein of another hepadnavirus.

9. A bacterial, yeast or mammalian cell line host transformed or, when the host is a mammalian cell line, transfected with a vector which comprises a DNA sequence encoding a chimaeric hepadnavirus core antigen protein including a foreign amino acid sequence comprising an epitope, the chimaeric protein being able to be expressed in the transformed or transfected host and the foreign amino acid sequence being inserted in or replacing all or part of an amino acid sequence from residue NLEDPASRDLV 75 to residue 85 when the core antigen protein is hepatitis B core antigen protein or a corresponding amino

acid sequence for a core antigen protein of another hepadnavirus.

10. A process for the preparation of particles composed of a chimaeric hepadnavirus core antigen protein which includes a foreign amino acid sequence comprising an epitope, the foreign amino acid sequence being inserted in or replacing all or part of an amino acid sequence NLEDPASRDLV from residue 75 to residue 85 when the core antigen protein is hepatitis B core antigen protein or a corresponding amino acid sequence for a core antigen protein of another hepadnavirus, which process comprises:

(i) culturing a bacterial, yeast or mammalian cell line host transformed or, when the host is a mammalian cell line, transfected with a vector which comprises a DNA sequence encoding the chimaeric protein, the chimaeric protein being able to be expressed in the transformed or transfected host, under such conditions that the chimaeric protein is expressed therein; and

(ii) recovering particles composed of the chimaeric protein which thus form.

11. A pharmaceutical or veterinary formulation comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as active ingredient, particles composed of a chimaeric hepadnavirus core antigen protein which includes a foreign amino acid sequence comprising an epitope, the foreign amino acid sequence being inserted in or replacing all or part of an amino acid sequence NLEDPASRDLV from residue 75 to residue 85 when

the core antigen protein is hepatitis B core antigen protein or a corresponding amino acid sequence for a core antigen protein of another hepadnavirus.

12. A formulation according to claim 11, wherein the foreign amino acid sequence is up to 40 amino acid residues in length.

13. A formulation according to claim 12, wherein the foreign amino acid sequence is up to 20 amino acid residues in length.

14. A formulation according to any one of claims 11 to 13, wherein the foreign amino acid sequence is inserted between hepatitis B core antigen protein residues AS corresponding to residues 80 and 81 or between corresponding residues of a core protein of another hepadnavirus.

15. A formulation according to any one of claims 11 to 14, wherein the epitope is the epitope of a pathogen.

16. A formulation according to claim 15, wherein the epitope is an epitope of hepatitis A virus, hepatitis B virus, influenza virus, foot-and-mouth disease virus, poliovirus, herpes simplex virus, rabies virus, feline leukaemia virus, human immunodeficiency virus type 1 or 2, simian immunodeficiency virus, human rhinovirus, dengue virus or yellow fever virus.

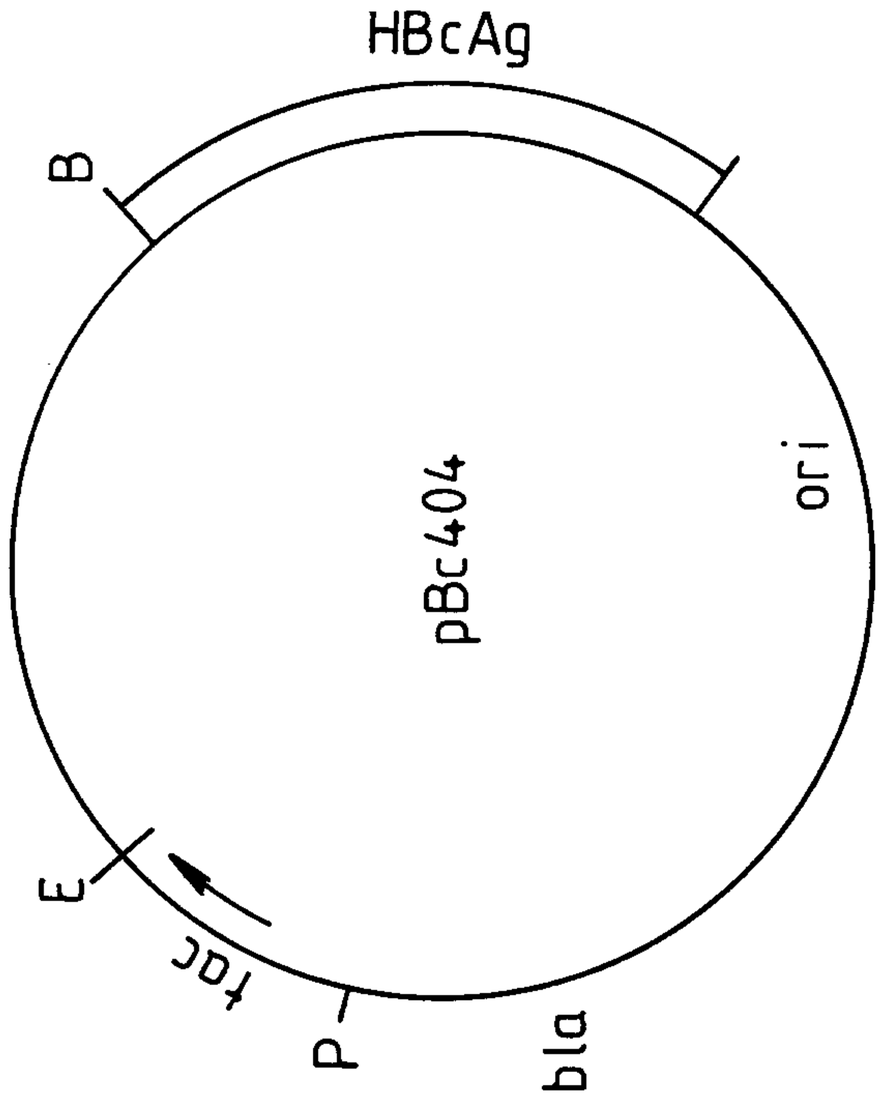
17. A formulation according to any one of claims 11 to 16, wherein a second said foreign amino acid sequence is fused to the N-terminal of the amino acid sequence of the core protein.

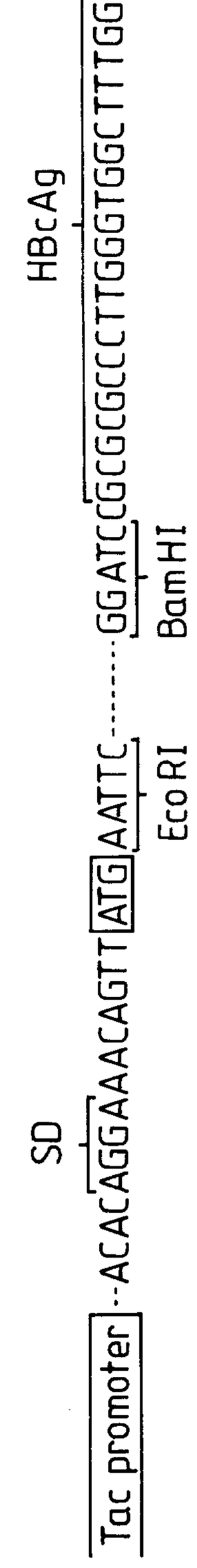
18. An expression vector which comprises a DNA sequence encoding a hepadnavirus core antigen and which has (a) a restriction site within a sequence NLEDPASRDLV encoding hepatitis B core antigen between amino acid residues 75 to 85 or a corresponding sequence of a core antigen protein of another hepadnavirus or (b) two restriction sites flanking the sequence as described in (a) or a part thereof.

19. A vector according to claim 18, wherein the restriction site (a) occurs between amino acids AS at amino acid residues 80 and 81 of the sequence encoding hepatitis B core antigen or at a corresponding sequence of a core protein of another hepadnavirus.

20. A vector according to claim 18, which is pPV-Nhe (NCIMB 40210) or pPN2 (NCIMB 40312).

Fig. 1.





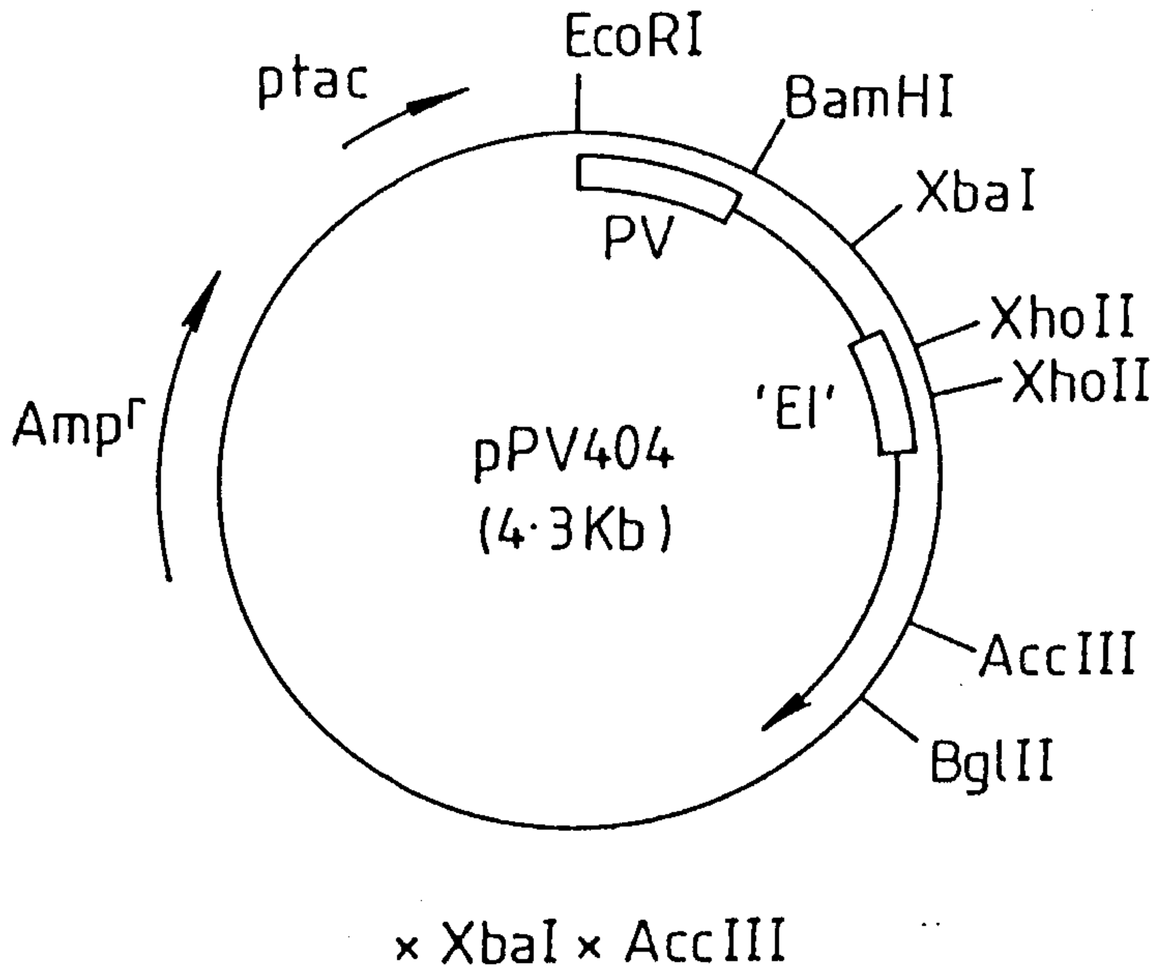
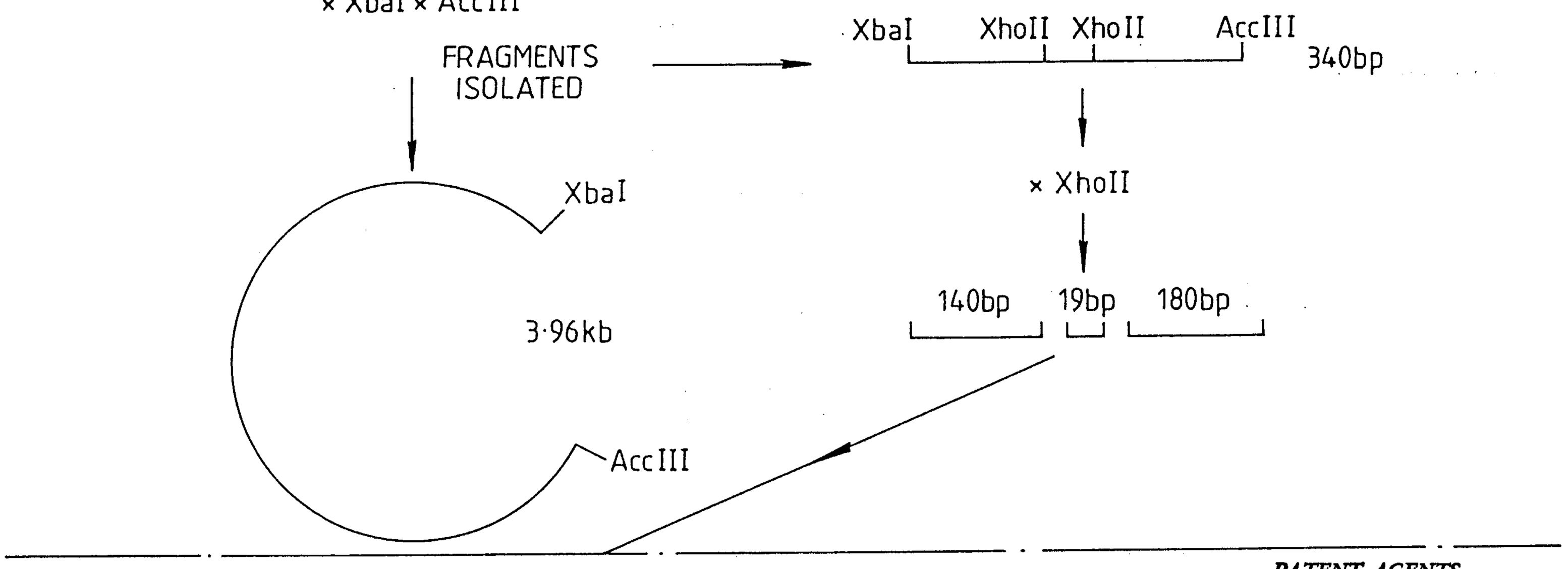


Fig. 2.



PATENT AGENTS

Swabey Ogilvy Renault

Fig.2(cont.)