Presentation and immunogenicity of viral epitopes on the surface of hybrid hepatitis B virus core particles produced in bacteria


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We recently reported the enhanced immunogenicity of a peptide epitope when it was presented as a fusion protein with hepatitis B core antigen. In those experiments the fusion protein was expressed in vaccinia virus. We have now refined the system so that large amounts of highly immunogenic particles can be produced using a simple bacterial expression system.

We describe the expression of three different viral epitopes as chimeric particles that induce good antibody responses to each epitope after one dose of low amounts of antigen. Finally we demonstrate that the immunogenicity is a reflection of both T helper cell sites within the core protein and also the particulate nature of the immunogens.

Introduction

Considerable progress has been made in recent years towards the development of subunit vaccines against viral diseases of both medical and agricultural importance. The ability to identify and readily synthesize components of viral particles that may be candidates for generating protective immune responses against a particular pathogen has focused attention on the ways in which these epitopes can be optimally presented to the immune system. The incorporaton of epitopes into complex oligomeric structures such as hepatitis B core (HBcAg) (Clarke et al., 1987; Stahl & Murray, 1989), or surface antigen (HBsAg) (Delpeyroux et al., 1986, 1988; Valenzuela et al., 1985) particles, the Ty element of yeast (Adams et al., 1987), or poliovirus virions (Burke et al., 1988; Martin et al., 1988) has shown particularly encouraging results. We recently reported the expression of a major immunogenic epitope from foot-and-mouth disease virus (FMDV) fused to the amino terminus of HBcAg (Clarke et al., 1987). The chimeric particles produced were highly immunogenic, inducing protective levels of antibody in laboratory animals almost as efficiently as the native FMD virion itself. Unfortunately we were unable to express this particular fusion protein in a bacterial system due to toxicity of the product to the host cell. However, small amounts of chimeric particles were produced using a vaccinia virus expression system. This expression system is not designed for the generation of large amounts of antigen and problems associated with the use of vaccinia virus restrict the applicability of this approach. To improve the versatility of this approach to antigen delivery we have now developed a simple bacterial expression system and report on the production of a number of chimeric particles carrying specific viral epitopes which, when administered to laboratory animals, produced excellent antibody responses. We also show that physical disruption of the particles decreases the immunogenicity considerably. This indicates that the nature of the immune response to the chimeric particles is a reflection of both the well characterized T helper cell sites within the core protein (Milich et al., 1987) and also the particulate nature of the antigen.

Methods

Construction of recombinant plasmids. The system used was an engineered plasmid vector pBC404 (pAT153-based) with gene expression being driven by the tac promoter (de Boer et al., 1982). A detailed map of the expression vector is shown in Fig. 1(a). The plasmid was engineered so that RNA transcripts produced from the tac promoter initiated translation at an AUG codon adjacent to a unique EcoRI restriction site. A second unique site, BamHI, was inserted upstream of a gene coding for HBcAg, which had been isolated from CsCl-purified Dane particles (adv serotype). This BamHI linker is actually located within a pre-core signal sequence that normally leads to secretion of the core protein from the cell (Ou et al., 1986; Roossinck et al., 1986; Uy et al., 1986; McLachlan et al., 1987). The nucleotide sequences in the region adjacent to the EcoRI and BamHI sites, as well as the translational reading frames, are indicated in Fig. 1(a).

To construct chimeric fusion particles coding for specific viral epitopes, synthetic oligonucleotides with cohesive ends for EcoRI (5') or BamHI (3') were prepared using an Applied Biosystems 381A DNA synthesizer (sequences available on request). These oligonucleotides were ligated into EcoRI–BamHI-digested pBC404, which had been
purified from low melting point agarose by standard methods. *Escherichia coli* strain JM101 (Messing, 1979) was then transformed with this DNA and recombinant plasmids were analysed by digestion of small-scale DNA preparations with restriction enzymes (Holmes & Quigley, 1981).

**Induction and purification of fusion proteins.** Bacteria harbouring recombinant plasmids were grown overnight in L-Amp medium to high cell density and diluted with fresh L broth (1:10) the following day. When the culture reached A560 = 1, expression was routinely induced by the immediate addition of isopropyl-β-D-thiogalactoside (IPTG; 60 μg/ml final concentration) and the bacteria were allowed to replicate for a further 6 to 8 h at 37 °C. Bacteria were then harvested and chimeric core particles purified as previously described (Stahl et al., 1982).

**Analysis of expressed products.** Bacterial lysates were initially analysed on 12.5% SDS–polyacrylamide gels (Laemmli, 1970), with samples representing the equivalent of 0.1 A560 of bacteria added to each lane. Proteins were visualized by Coomassie blue staining and also by Western blotting (Towbin et al., 1979), using either anti-HBcAg–specific sera or anti-peptide sera for the particular epitope. Blots were developed by incubation with anti-species horseradish peroxidase conjugate (Miles-Yeda) and visualized using diaminobenzidine tetrahydrochloride (Sigma) and hydrogen peroxide.

**Synthetic peptides and their antisera.** All HBcAg fusion proteins were screened using anti-peptide antisera to the added rhinovirus, poliovirus or feline leukaemia virus (FeLV) epitopes. Peptides to each epitope were synthesized using an adaptation of the Merrifield (1963) technique, described by Houghten (1985). Each peptide had an additional non-natural cysteine residue at its carboxy terminus to facilitate coupling to keyhole limpet haemocyanin (KLH) via an m-maleimidobenzoic acid N-hydroxysuccinimide ester.

Coupled peptides were inoculated intramuscularly into groups of four guinea-pigs using a 200 μg dose in incomplete Freund’s adjuvant (IFA). All animals were boosted with a similar inoculum at 42 days and sera were collected 28 days later. The sera were tested for anti-peptide activity, which was generally 1:10 000 or greater in an indirect ELISA, and used as reagents for testing fusion proteins.

**ELISA.** Anti-peptide, anti-virus and anti-HBcAg activity in serum samples was measured using a modification of the indirect or double antibody sandwich ELISA method (Voller et al., 1976). In the sandwich ELISA human anti-HBcAg antibody (1:200) was used to trap 2 μg/ml HBcAg onto the plate, whereas in the indirect ELISA 2 μg/ml peptide or virus was coated directly onto the plastic. The plates were then washed and test serum samples at a range of 0.5 log10 dilutions from 10−1 were added. After incubation for 1 h at 37 °C plates were washed and anti-guinea-pig 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 0.1 M phosphate–0.05 M-citrate buffer) was added. The resulting colour development was stopped with 12.5% (v/v) sulphuric acid after 5 to 7 min and the A492 was measured in a Titertek Multiskan (Flow Laboratories).

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

**Detection of particulate structures in bacterial lysates.** Bacterial lysates showing expressed fusion proteins were examined for the presence of assembled core particles. Prepared lysates (3 ml) were loaded directly onto (34 ml) 15 to 45% linear sucrose density gradients and centrifuged at 100 000 g for 4 h at 20 °C in a Beckman SW28 rotor. Gradients were fractionated into 2 ml aliquots, which were analysed spectrophotometrically at 260 nm. This assay relies on the presence of nucleic acid within the assembled core particles. The peak fractions from the gradient were pooled, dialysed against phosphate-buffered saline (PBS) to remove sucrose and the particles were concentrated by centrifugation (100 000 g, 4 °C in an SW28 rotor for 16 h). The pellets particles were then resuspended in PBS and formulated for vaccine preparation.

**Disruption of native core particles.** Denaturation of the chimeric core particles into subunit proteins was achieved following the method of Mackay et al. (1981). Twenty mg of purified particles was treated with 0·1% SDS, and 0·1% 2-mercaptoethanol for 1 h at 37 °C. Under these conditions sucrose gradient centrifugation showed the presence of a small residual amount of particulate matter. To ensure complete removal of these particles the disrupted sample was further centrifuged for 1 h at 200 000 g in an SW50 rotor. The supernatant was then used directly for vaccine formulation.

**Animal inoculations.** Groups of four female Dunkin Hartley guinea-pigs weighing approximately 400 g were each inoculated intramuscularly with a 0·5 ml dose of the experimental HBcAg fusion protein preparations formulated in IFA. Further details of antigen doses, inoculation schedules and sampling for individual experiments are given in the Results section.

**Results**

**Expression of chimeric particles**

The plasmid vector designed to express chimeric HBcAg fusion particles is shown in Fig. 1 (a). Initially three sequences were chosen from viral genomes that were predicted to code for important immunogenic regions on the parent virion. These amino acid sequences are shown in Fig. 1 (b). The C3 epitope from poliovirus type 1 (Mahoney) (Kitamura et al., 1981; Racaniello & Baltimore, 1981), designated PV-1, was chosen because it is a small, well characterized linear epitope (Horaud et al., 1987); monoclonal antibodies (MAbs) specific for the epitope are available and it has been used as the target sequence in other particulate fusion systems (Charbit et al., 1988; Delpeyroux et al., 1986, 1988). The second epitope we used was derived from human rhinovirus type 2 (HRV-2) and represented a site on viral protein 2 (VP2) that had been shown to be antigenic (Skern et al., 1987) and to induce virus-neutralizing antibodies when presented as a linear peptide (Francis et al., 1987). Finally, the third epitope that we describe represents a predicted immunogenic region from the surface glycoprotein of FeLV, which was suggested to us by Dr J. Neil and Professor O. Jarrett (University of Glasgow, U.K.).

Synthetic oligonucleotides coding for these sequences were inserted into the bacterial expression vector pBC404. Constructs were designed with internal unique restriction sites allowing simple screening of recombinant colonies. Characterized clones were initially used on a small scale to produce recombinant antigen and samples from cell lysates of these bacteria were analysed by SDS–PAGE and Western blotting. Fig. 2 (a) shows a
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Fig. 1. (a) Schematic representation of expression plasmid pBC404. The exact nucleotide sequence surrounding the EcoRI-BamHI cassette region as well as the translational reading frame are shown. The ATG codon used for translation initiation is boxed. E, EcoRI; B, BamHI; P, PstI; ori, origin of replication; bla, β-lactamase; SD, Shine-Delgarno sequence. (b) Amino acid sequences of the amino-terminal extensions to HBcAg in each fusion protein. Sequences coding for the heterologous viral epitope are boxed. The sequence for PV-1 (Mahoney) was taken from Kitamura et al. (1981) and Racaniello & Baltimore (1981), for FeLV-A from Stewart et al. (1986) and for HRV-2 from Skern et al. (1985).

Coomassie blue-stained gel from two individual colonies harbouring the PV-1–HBcAg plasmid. A novel protein of the expected Mr (24K) was expressed in the recombinants (lanes 1 and 3), which was not present in the parent bacterial strain (lane 5). This protein is also present in the uninduced recombinants (although at a lower level) and presumably results from 'leaky' expression of the tac promoter in this particular strain of E. coli (JM101). The induced protein reacted with both anti-HBcAg (Fig. 2b) and anti-PV-1 peptide (Fig. 2c) sera in Western blots, confirming the presence of epitopes from both viruses on the same recombinant fusion protein. Similar results were obtained with both the FeLV and HRV-2 epitopes (results not shown). Expression levels, as shown in Fig. 2(a), were consistently good for each construct, with yields of 50 to 80 mg of protein from 1 l of culture.

Having established the presence of fusion proteins carrying the correct epitopes, bacterial lysates were examined for the presence of assembled core particles. Prepared lysates were centrifuged through sucrose density gradients and the fractions analysed spectrophotometrically at $A_{260}$ for the presence of nucleic acid within core particles and also by ELISA for the presence of HBcAg. Fig. 3(a) shows the gradient profile produced by the PV-1–HBcAg lysate and, as expected, the ELISA and $A_{260}$ peaks coincided. Subsequent SDS–PAGE of the gradient fractions (Fig. 3b) revealed the presence of the induced protein (24K) within the gradient peak. Interestingly, a second band of approximately 18K also copurified with the particles. This protein was subsequently found to represent a carboxy-terminal cleavage product of the PV-1–HBcAg protein, which reacted with anti-PV-1 peptide serum by Western blotting (not shown). Material present in the peak fractions from the gradient was pooled, dialysed to remove sucrose, concentrated by centrifugation and used for further characterization and immunogenicity studies.

Characterization of chimeric particles

Preliminary characterization of each antigen was performed using a sandwich ELISA in which particles were trapped on microtitre plates with human anti-core serum.
and then screened for the presence of anti-peptide or anti-core reactive epitopes on the surface of the particles using the corresponding guinea-pig antiserum. Fig. 4 shows that PV-1-HBcAg extracts reacted with both anti-PV-1 C3 peptide and anti-HBcAg sera, whereas control native bacterially expressed HBcAg reacted only with the anti-HBcAg serum. Similar results were observed with the FeLV and HRV-2 epitopes.

Further characterization of the PV-1-HBcAg was facilitated by the availability of MAbs specific for the PV-1 epitope. ELISA of this antigen indicated that an anti-C3 (Mahoney) MAb (11) was able to detect the C3 epitope on the surface of the particles (Fig. 5). A similar MAb (955), which is specific for the same region from PV-1 Sabin (Burke et al., 1988) and does not react with PV-1 Mahoney, did not react with the particles. The sequence differences in this region between PV-1 Sabin (Nomoto et al., 1982) and PV-1 Mahoney (Kitamura et al., 1981; Racaniello & Baltimore, 1981) are limited to two amino acid substitutions. Therefore the epitope as presented on the surface of PV-1-HBcAg particles behaved as in the native virion.

**Immune response of laboratory animals after inoculation with the particles**

Immunogenicity studies were carried out for each antigen by intramuscular injection of guinea-pigs using IFA. In the case of the FeLV epitope three groups of animals were injected with antigen corresponding to 15, 1.5 or 0.15 µg of the heterologous epitope. For the other antigens only the two lower doses were used. These doses were based on the results of the immunogenicity of chimeric particles carrying FMDV epitopes (Clarke et al., 1987). In each case animals were boosted with a second dose of the same amount of antigen, also in IFA, at 56 or 72 days. Blood samples were removed from individual animals at approximately 14 day intervals and assessed for the presence of anti-HBcAg, anti-peptide epitope or (in the case of the HRV-2 epitope) anti-virus antibodies. The results for the immunogenicity studies are shown in Tables 1 to 3. In the 14 day sera the immune response against the core protein was extremely high for each construct, even at the lowest dose. The titre continued to rise to a plateau level by 28 days, which
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Fig. 3. Bacterial lysates containing HBCAg and PV-1 reactive antigens analysed by sucrose density gradient fractionation. Individual fractions were assessed for the presence of nucleic acid and ELISA reactive material (a) at A_260 (○) and A_450 (●) and also by SDS-PAGE on 12.5% gels with Coomassie blue staining (b). Fraction 14 corresponds to the top of the gradient, fraction 1 to the bottom.

persisted until the animals were boosted with a secondary injection, after which an elevated titre could be measured in some groups. Responses to the peptide epitope, although significant, were not as high as those to the core antigen. The anti-peptide levels also differed between the three constructs with the HRV-2 and FeLV epitopes giving better responses than the PV-1 sequence. Most importantly the HRV-2 anti-peptide response was matched by the anti-virus activity as measured by ELISA. In general the bacterially expressed chimeric particles were as immunogenic as those we had previously produced using a vaccinia virus system and

Fig. 4. Sandwich ELISA of HBCAg and PV-1-HBCAg particles trapped with human anti-HBCAg antiserum and detected with guinea-pig antisera: anti-HBCAg with PV-1-HBCAg (●) or HBCAg (▲) and anti-PV-1 peptide with PV-1-HBCAg (●) or HBCAg (○).

Fig. 5. Sandwich ELISA of PV-1-HBCAg particles trapped with human anti-HBCAg serum and detected with mouse MAb 955 (▲), or 955 (▲), or normal mouse serum (●).
Table 1. Response of guinea-pigs to HBcAg–FeLV particles

<table>
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<tr>
<th>Group no.</th>
<th>Dose of fusion protein (µg)</th>
<th>Peptide dose (µg)</th>
<th>ELISA antigen</th>
<th>Days after initial inoculation*</th>
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<td>200</td>
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</tr>
<tr>
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<td>2</td>
<td>0:17</td>
<td>FeLV peptide</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Core</td>
<td>&lt;1:0†</td>
</tr>
</tbody>
</table>

* Secondary inoculation was at day 56.
† log10 mean ELISA endpoint titre.

Table 2. Response of guinea-pigs to HBcAg–HRV particles

<table>
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<th>Group no.</th>
<th>Dose of fusion protein (µg)</th>
<th>Peptide dose (µg)</th>
<th>ELISA antigen</th>
<th>Days after initial inoculation*</th>
</tr>
</thead>
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<td>HRV-2 virus</td>
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<td></td>
<td></td>
<td>Core</td>
<td>&lt;1:0†</td>
</tr>
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<td>HRV-2 virus</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Core</td>
<td>&lt;1:0†</td>
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</tbody>
</table>

* Secondary inoculation was at day 56.
† log10 mean ELISA endpoint titre.

Table 3. Response of guinea-pigs to HBcAg–PV-1 particles

<table>
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<th>Group no.</th>
<th>Dose of fusion protein (µg)</th>
<th>Peptide dose (µg)</th>
<th>ELISA antigen</th>
<th>Days after initial inoculation*</th>
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<td>Core</td>
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</tr>
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* Secondary inoculation was at day 56.
† log10 mean ELISA endpoint titre.

represented a powerful means of potentiating an immune response against a heterologous epitope.

Immunogenicity of denatured HBcAg–HRV-2 particles

Detailed studies into the mechanism of the immune response against HBcAg (Milich & McLachlan, 1986) have revealed that the particles are able to induce both T helper cell-dependent and -independent responses. The T cell-dependent response has been shown to reflect the presence of several potent T helper cell-stimulating sequences in the core protein, which are functional in defined strains of mice. Conversely the T cell-independent nature of the response may be due to the repeating nature of epitopes on the surface of the particle. We were therefore interested to determine the contribution of the T helper cell sites and the repeating nature of the heterologous epitope towards the immune response against the chimeric particles. We examined this with the HRV-2 chimera by administering the antigen as
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3.0
t(0)
1-0
10 20 30 40 50 60
2.0
1-0
10 20 30 40 50 60
Inoculation
ELISA endpoint titre (log10)

(a)
(b)

Fig. 6. Indirect ELISA of pooled guinea-pig blood samples taken at the days indicated post-inoculation. Assays were carried out using either a nine amino acid synthetic peptide (a) corresponding to the HRV-2 epitope, bound directly to the solid phase or HRV-2 virions (b). Intact fusion protein at 20 µg (●) or 2 µg (○) and disrupted fusion protein (▲) were used.

intact particles, or as denatured free antigen. The immune responses of these antigen preparations in guinea-pigs are shown in Fig. 6. Both anti-peptide and anti-virus titres were significantly reduced (by approximately 100-fold) when the particles were disrupted and 2 µg of intact particles gave consistently higher ELISA endpoint titres than 10-fold more disrupted antigen. Even so, the observation that responses were obtained against the HRV-2 epitope (which is non-immunogenic as a free peptide) on the disrupted particles indicates that the T helper cell sites within the core protein help in inducing B cell responses against the added epitope.

Discussion

In this paper we describe the synthesis of three recombinant proteins composed of viral epitopes fused to the amino terminus of HBcAg. The fusion proteins are expressed to high levels in a bacterial system and spontaneously assemble into particulate structures, as has been shown for the native core antigen (Stahl et al., 1982). Because of the predicted interaction between the carboxy terminus of HBcAg with nucleic acid in the virion core (Pasek et al., 1979) we made our fusions to the amino terminus. However, it has been reported that deletion of the carboxy terminus of the antigen does not abolish particle morphogenesis and, indeed, fusion of peptide epitopes to the carboxy terminus has recently been reported (Stahl & Murray, 1989). A recent model of the structure of the core antigen has predicted that both the amino and carboxy termini of the protein are internal (Argos & Fuller, 1988). In spite of this it is clear from this study and others that fusion of heterologous epitopes to either the amino (Clarke et al., 1987; this paper) or carboxy termini (Stahl & Murray, 1989) results in presentation of the epitope on the surface of the particle. This would therefore suggest that the amino terminus of the authentic protein must reside relatively near the surface of the particle in order for the amino-terminal extensions to be exposed.

The particulate structure of the antigen allows easy purification of the hybrid particles by sucrose gradient centrifugation. Analysis of the purified particles routinely shows the presence of the authentic fusion protein and a smaller product, which also reacts with anti-peptide sera in Western blots. This smaller product represents a carboxy-terminal deletion of the protein, as occurs in the natural product HBeAg. Experimental evidence indicates that HBeAg may be produced by proteolytic self-cleavage of the core protein (Miller, 1987), which may involve an aspartate protease active site motif located towards the amino terminus of the protein. However, it has recently been reported that site-directed mutagenesis of this motif has no effect on the conversion of HBcAg to HBeAg (Nassal et al., 1989).

Regardless of the mechanism of this degradation the fact that it occurs has important implications for the use of HBcAg as an immunological presentation system. Although the natural form of HBeAg is as soluble dimers of 17K subunits (Stahl & Murray, 1989), expression of truncated forms of HBcAg in E. coli corresponding to HBeAg results in the formation of particulate structures similar to HBcAg particles. However, it has recently been shown that authentic HBcAg particles are significantly more immunogenic than particulate or non-particulate forms of HBeAg (Milich et al., 1988). This disparity has been explained by the observation that HBcAg can function as a T cell-independent antigen, whereas HBeAg is T cell-dependent, even when presented as a particle (Milich & McLachlan, 1986). The autocatalytic cleavage of the core protein within the
particle may therefore have significant effects on the immunogenicity of the chimeric particles.

Interestingly, the degree of cleavage between different preparations is somewhat variable. This may relate to the observation that there is a critical concentration requirement for aspartate protease molecules before efficient autocatalytic processing can occur (Krausslich et al., 1988). Experiments to understand the mechanism of this cleavage and to control it are in progress. Unfortunately it is difficult to compare the immunogenicity data presented for the carboxy-terminal (i.e. HBeAg) fusion particles of Stahl & Murray (1989) because all immunizations were carried out in Freund's complete adjuvant (FCA), which will tend to mask the genuine immunogenicity of the particles. To address this question we are in the process of moving some of our epitopes from the amino terminus to the truncated carboxy terminus and other parts of the protein to compare immune responses.

The immunogenicity results presented here demonstrate the potent immunogenicity of the HBcAg within the fusion proteins using single doses of very small amounts of material in IFA. The FeLV and HRV-2 fusion particles elicited high levels of antibody to the added epitope, as we had previously shown with FMDV–HBcAg particles (Clarke et al., 1987). Unfortunately, antibodies to the predicted FeLV epitope did not recognize the virus, whether they were elicited by core fusion particles or KLH-coupled synthetic peptides. However, the HRV-2 epitope that has been shown (Francis et al., 1987; Skern et al., 1987) to represent a linear antigenic site on the virion induces anti-virus antibodies when administered as the HRV–2–HBcAg particle (Fig. 6). This fusion protein has been used for detailed qualitative and quantitative comparison of the immune response compared to free or KLH-coupled peptide (Francis et al., 1990). In dose–response experiments the immune response against the HRV-2 epitope was approximately 10-fold greater when presented on HBcAg particles than on KLH.

The responses to the PV-1 epitope were lower than those elicited by the other epitopes. This may be because at nine amino acids the epitope is too small to be efficiently exposed on the surface of the particle. One of our aims in using the PV-1 C3 epitope was to assess the relative merits of HBcAg as a presentation system, compared with other methods of presentation that have been used for this epitope. Most of the data reported have been generated from HBsAg chimeras and disappointingly low anti-PV-1 neutralizing titres were generated by a homogeneous HBsAg–PV particle, despite the fact that the antigen was administered in FCA (Delpeyroux et al., 1986). Some improvement was observed in rabbits when mosaic particles composed of both HBsAg–PV and HBsAg were used, but a variable response was observed between individual animals and, again, vaccines were formulated in FCA (Delpeyroux et al., 1988). In view of the fact that anti-peptide titres were not reported for the HBsAg constructs and that the HBsAg–PV-1 and HBCAg–PV-1 chimeras were administered to different species under differing vaccination regimes it is difficult to make conclusions on the relative merits of the two systems. A controlled experiment to compare the two systems directly is now in progress.

Finally, the observation that deliberate denaturation of the HRV-2–HBcAg particles results in a considerable, although not total, loss of immunological activity to a peptide that itself is not immunogenic (Francis et al., 1987) suggests that the immune response is composed of a component due to the particulate nature of the antigen and a component from the T helper cell stimulatory activity of the HBcAg protein. Milich et al. (1988) have shown using a dinitrophenol hapten chemically linked to HBcAg that a T cell-independent response was obtained against the hapten moiety in athymic mice. However, it has also been shown (Milich et al., 1988) that the particulate structure alone is not sufficient to confer T cell independence and additional factors are likely to be important (Dintzis et al., 1983). We have therefore established that a T cell-independent response can be raised against a peptide epitope using the HRV-2–HBcAg particles as a model system in nude mice (Francis et al., 1990).

Finally, it is important to emphasize that this system is primarily designed for the improved presentation of sequential determinants. The use of the system for the presentation of conformational determinants requires further sophistication, such as the insertion of epitopes into immunodominant regions within the protein. Preliminary experiments along these lines are encouraging (B. E. Clarke et al., unpublished).

We thank Dick Campbell for the synthetic peptides, Morag Ferguson for the gift of PV-1 monoclonal antibodies, Tony Carroll for synthetic oligonucleotides and Lorraine Joyce for typing.

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Clarke, B. E., Newton, S. E., Carroll, A. R., Francis, M. J., Appleyard, G., Syred, A., Highfield, P., Rowlands, D. J. &
Hybrid hepatitis B core particles


(Received 4 November 1989; Accepted 10 January 1990)