Strain Variability and Localization of Important Epitopes on the Major Structural Protein (VP2) of Infectious Pancreatic Necrosis Virus

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Infectious pancreatic necrosis virus (IPNV), a birnavirus, is an important pathogen in fish farms. Analyses of viral proteins showed that VP2 is the major structural and immunogenic polypeptide of the virus. All neutralizing monoclonal antibodies (mAbs) against VP2 are specific to VP2 and bind to continuous or discontinuous epitopes. In order to determine which parts of the protein are involved in antigenic variations, five IPNV strains were sequenced over the VP2 coding region. Comparison of the sequences obtained with three previously published strains revealed a central variable domain (positions 183 to 335) which encompasses two hydrophilic hypervariable segments. Viral mutants which escaped neutralization were then selected with anti-VP2 mAbs directed against discontinuous epitopes. Sequencing of three mutants revealed a single amino acid mismatch in each of them. All of these substitutions occurred in the hypervariable segments, suggesting that these regions are involved in the formation of a discontinuous epitope. Finally, expression of different truncated VP2s in Escherichia coli allowed localization of the binding site for neutralizing mAbs which recognize continuous epitopes. One of these mAbs bound to the region adjacent to the C-terminus of the variable domain of VP2, while two others reacted with the central and C-terminal parts of the variable domain. No antibody reacted with the N-terminus of VP2. These results suggest that the variable domain of VP2 and the 20 adjacent amino acids of the conserved C-terminal part are the most important in inducing an immune response for the protection of animals.

INTRODUCTION

Infectious pancreatic necrosis is one of the major viral diseases of juvenile trout and salmon (Pilcher and Fryer, 1980; Wolf, 1988; Meyer, 1991). The causal agent of this contagious, high-mortality disease is infectious pancreatic necrosis virus (IPNV), which belongs to the Birnaviridae family (Francki et al., 1991). The major structural and immunogenic proteins of the virus, VP2 and VP3, are produced by cleavage of the amino- and carboxy-terminal parts, respectively, of a precursor polyprotein encoded on genome segment A (Huang et al., 1986; Duncan et al., 1987; Nagy et al., 1987), which is the largest of the two dsRNA segments of IPNV (Dobos and Roberts, 1983). VP2 (54 kDa) is associated with the outer capsid and is the late maturation cleavage product of preVP2 (62 kDa). Both forms of the polypeptide are usually found in purified virus preparations and together they represent 64% of all virion proteins (Dobos et al., 1991). All the epitopes (continuous and discontinuous) recognized by neutralizing monoclonal antibodies (mAbs) are located on VP2 (Caswell-Reno et al., 1986; Nagy and Dobos, 1987; Christie et al., 1990; Tarrab et al., 1993, 1995). VP3 (29 to 31 kDa) is thought to be an internal protein of the virus (Dobos et al., 1977) and contains some important continuous nonneutralizing epitopes (Caswell-Reno et al., 1986; Christie et al., 1990; Tarrab et al., 1993).

Most of the information available on the antigenic domains of birnavirus proteins comes from studies on infectious bursal disease virus (IBDV). These works showed that a central variable region of IBDV VP2 contains the conformational epitopes recognized by neutralizing mAbs (for review, see Müller et al., 1992). It is suspected that IPNV has the same antigenic structure. Comparison of the N1 and Jasper strains of IPNV revealed that, as for IBDV, the N- and C-terminal parts of IPNV VP2 are more conserved than the central region of the protein (Håavarstein et al., 1990). Moreover, Tarrab et al. (1995) showed evidence for a major, conformation-dependent, and immunodominant domain of VP2, where continuous epitopes could represent portions of a larger discontinuous epitope. The exact size of this region has not yet been determined, though it was shown to be located in the central third of the protein (Frost et al., 1995; Liao and Dobos, 1995).

The two birnaviruses share many similar features, but IPNV strains show more genomic variations than those of IBDV (Kibenge et al., 1991; Heppell et al., 1993). The proposed serological classification of IPNV also appeared more complex, with two serogroups, A and B, and up to 10 serotypes inside serogroup A (Hill and Way, 1983, 1988; Christie et al., 1988, 1990; Caswell-Reno et
Viral strains used in this work [LWVRT 60-1 (VR-299), d'Honnincthun (Fr.21), Ab. Hecht (He), and Canada 2 (C2)] were obtained from the American Type Culture Collection and are related to serotypes A1, A2, A3, A4, and A7, respectively. All viruses were propagated on CHSE-214 cells and concentrated by ultracentrifugation of the clarified supernatant, as previously described (Heppell et al., 1992). Viral dsRNA was then extracted with phenol and chloroform, followed by digestion with proteinase K (Heppell et al., 1992).

Cloning and sequencing

The preVP2 coding region on genome segment A was reverse transcribed and amplified by PCR following the protocol described elsewhere (Heppell et al., 1992). Oligonucleotides IPN-0 (5′-CTCTCATGACATGAGCACAC-CCAAGGCAACCACA-3′) and IPN-7 (5′-GGTGCTAGTATGACATGACTC-3′), which contain an added XbaI restriction site (underlined characters) and the complement of a stop codon (boldface characters in IPN-7), were synthesized according to the published sequence of the Jasper strain (Duncan and Dobos, 1986). The position of the start codon of the polyprotein is indicated by boldface characters in IPN-0. These primers allowed the amplification of a 1594-bp cDNA fragment (nucleotides 144 to 1737) for all strains except Hecht. For this strain, the oligonucleotide LAC-5 (5′-GAGACGC-TCACAGGACC-3′) was used instead of IPN-0 in order to amplify a portion of preVP2 (nucleotides 386 to 1737).

The amplified fragments were purified with the Gene-Clean kit (Bio 101) and cloned directly into the pCR II vector (Invitrogen). Inserts were then sequenced using an A.L.F. DNA sequencer (Pharmacia) or traditional di-deoxynucleotide chain termination procedure (Sanger et al., 1977). For each virus strain, three fragments, obtained from independent RT-PCR reactions, were cloned and sequenced in both directions. In cases where a nucleotide mismatch was detected in one of the three fragments, only the two identical fragments were considered to determine the consensus sequence at this particular position. The sequence of the missing portion of the preVP2 of Hecht (nucleotides 144 to 385) and the region upstream of nucleotide 144, for all strains (5′ end to nucleotide 143), was determined previously (Heppell et al., 1995). Sequences were analyzed with MacVector release 4.5.0 (Eastman Kodak) and GeneWorks release 2.4 (Intelligenetics Inc.) programs.

Expression and detection of truncated VP2

For the expression of VP2 as a fusion protein, one of the three fragments obtained previously for the VR-299 strain was transferred from the pCR II vector to the pTOPE-1b(+) expression vector (Novagen), downstream of and in frame with the T7 gene 10 included in the plasmid. The preVP2 coding sequence was then truncated at different restriction sites, as indicated in Fig. 1, and the shortened plasmids were closed with T4 DNA ligase. For the expression of full-length (i.e., entire preVP2) and truncated VP2, pTOPE-1b(+) recombinant vectors were inserted into Escherichia coli Novablaue (DE3) cells (Novagen). Bacterial cultures were grown at 37°C until the O.D. 600 reached 0.5, and then they were induced with 0.4 mM IPTG for 2 hr at 37°C. Volumes of 1 ml of the cell cultures were centrifuged for 5 min and the pellets were resuspended in 200 μl of SDS-PAGE sample buffer. For each clone, 20 μl of these bacterial lysates was loaded on a 10% polyacrylamide gel. A concentrated virus suspension (strain VR-299), prepared as described above, was used as positive control. In this case, 5 μl of the virus preparation was mixed with 15 μl of SDS-PAGE sample buffer before loading on the gel.

After electrophoresis, proteins were stained with Coomassie blue or transferred onto PVDF membrane (Bio-Rad). In the latter case, they were probed with a rabbit anti-VR-299 serum or with anti-VP2 mAbs. Monoclonal antibodies used in this work (LW4, LW6, LW7, LW9, and LW10) were raised against the VR-299 strain and have been characterized in our laboratory (Tarrab et al., 1993). They all neutralize virus infectivity and bind to continuous (LW4, LW6, and LW7) or discontinuous (LW9 and LW10) epitopes. Detection was carried out with a HRP-conjugated anti-rabbit IgG or anti-mouse IgG and a chemiluminescence Western blotting kit (Boehringer-Mannheim).

Selection of escape mutants

Monoclonal antibodies LW9 and LW10 were used to select escape mutants in order to locate amino acid resi-
dues that are potentially involved in the formation of discontinuous epitopes on VP2. The VR-299 strain was pre-incubated with one of the two mAbs for 1 hr and then inoculated onto CHSE-214 cells in the presence, in the overlay agarose, of the same mAb used for selection (Öppling et al., 1991). Individual plaques were taken and grown following the same procedure at least five times. Genomic dsRNA was finally extracted, cloned, and sequenced as described previously.

RESULTS

Comparison of the sequences obtained

Deduced amino acid sequences obtained from the five IPNV strains are shown in Fig. 2, alongside previously published sequences. Analysis of these data revealed that strains Fr.21 and C2 have an open reading frame (ORF) containing 17 additional residues at their N-terminal part. This is due to a frameshift mutation in a highly variable region of the nucleotide sequence located upstream of the methionine at position 1, which has already been identified (Heppell et al., 1995). The NH$_2$ extremity of the Hecht strain ORF also differed from the others in that it begins with two consecutive methionines and contains an additional residue at position 5. Except for these differences, other variations between strains were simple amino acid mismatches.

The central region of VP2 (positions 183 to 335) showed more variation than its extremities. In fact, 59.5% of the amino acids are conserved among all strains in this region, compared to 87.4 and 84.1% for the N- and C-terminal parts, respectively (positions 1 to 182 and 336 to 492). Residues 493 to the end were not considered in the comparison because they probably belong to the NS protein, which lies between VP2 and VP3 in the polyprotein (Duncan et al., 1987). Within the variable portion of VP2, many mismatches appeared clustered in two hypervariable regions: residues 243 to 261 and 275 to 288. Interestingly, these two regions correspond to small hydrophilic peaks separated by hydrophobic portion. These features, however, are not perfectly conserved on all viruses, particularly on strains C2 and He, which have a different hydrophilicity pattern in the hypervariable region nearest the N-terminus of the protein. Despite this, the overall hydrophilicity profile of VP2, calculated by the method of Kyte and Doolittle (1982), showed great similarity between strains (Fig. 3).

Percentages of homology between amino acid se-
FIG. 2. Deduced amino acid sequences obtained from the five IPNV strains used in this work and their corresponding portions on the published sequences of IPNV. Homology to the VR-299 strain is indicated by dots, and dashes stand for missing residues. The central variable domain of VP2 is underlined, while the two hypervariable segments are doubly underlined. Amino acid residues shown in lowercase letters probably belong to the NS protein, according to Duncan et al. (1987). Sequences of the N1 and DRT strains were obtained from Haavestain et al. (1990) and Chung et al. (1993), respectively. The sequence of the Jasper strain (Duncan and Dobos, 1986) is not presented because it is identical to VR-299.

Sequences were calculated (Table 1). Strains VR-299 and Jasper appeared identical at the amino acid and the nucleotide levels, while DRT was very similar to them, with 99.2% homology (at the amino acid level). This group of strains showed little variation compared to the second group which includes Fr.21 and N1 (98.6% homology), as well as C2 and Ab, although these last two strains are not so closely related. The Hecht strain would belong to
FIG. 3. Hydrophilicity profiles calculated for the five IPNV strains sequenced. Signs of the values (to the left) were inverted so that hydrophilicity was plotted instead of hydrophobicity as originally used by Kyte and Doolittle (1982). The approximate locations of the substitutions observed with the VR-299 escape mutants (small arrows) and of the variable domain of VP2 are indicated.

a third group since it showed less than 88.5% similarity with any other virus.

Expression of truncated proteins

Full-length and truncated VP2s of the VR-299 strain were produced in a prokaryotic expression vector as fusion proteins, in order to locate the binding site of mAbs in relation to the variable domain of the protein. For each bacterial clone, a polypeptide having the expected molecular mass (the VP2 portion plus its 29-kDa fusion partner) was observed on SDS–PAGE (Fig. 4A). Monoclonal antibodies LW4, LW6, and LW7, as well as the rabbit anti-VR-299 serum, reacted strongly against the full-length VP2 in bacteria and in the concentrated virus preparation used as positive control (Figs. 4C to 4F). A few smaller polypeptides were also detected in bacterial lysates. These were probably partially degraded fusion
proteins or were produced by an early termination of translation. Similar results were obtained by Lawrence et al. (1989) with a different prokaryotic expression vector. In the case of the anti-VR-299 serum, however, one of the lower bands in the concentrated virus preparation corresponded to VP3 (Fig. 4F). In addition to the whole VP2, antibodies also reacted with clone EM351, which lacks most of the conserved region of VP2 located downstream of the variable domain. Only mAb LW7 showed a significant decrease in the intensity of the signal obtained with this clone, when compared to EMVP2.

Clone EM331, which contains the entire variable domain and the N-terminal conserved region of VP2, was the most discriminant. The antiserum and mAbs LW6 and LW7 showed a weak reaction with this clone (compared to the reaction obtained with EMVP2), while mAb LW4 completely failed to recognize it. This suggests that the binding site of LW4 was located between amino acids 331 and 351.

No antibody reacted with the EM271 deletion clone but, surprisingly, a very weak signal was consistently obtained with the shorter clone, EM245, using the same antiserum. However, it has to be highlighted that the band appearing at the expected size for the fusion protein in EM245 was less intense than some of the nonspecific bands observed in Fig. 4F. Hence, the reaction between the antiserum and the fusion protein in clone EM245 was weak, although it seemed to be specific since it never appeared with the other clones.

When the entire variable domain was deleted (clones EM184 and EM75) no reaction was observed. This suggests that the N-terminal part of VP2 does not contain an important epitope. These results were confirmed by clone EMvar, which contains only the variable domain of the protein. The signal obtained with this clone (Fig. 4B) was identical, for all antibodies, to the one observed with clone EM331. Thus, the N-terminal region had no influence on the reaction.

The LW9 mAb, which binds to a discontinuous epitope, was also tested in Western blot, but results were negative. Monoclonal antibodies LW9 and LW10 also failed to react in an immunodot assay using bacterial lysates obtained in nondenaturing conditions (not shown).

Detection of mismatches in escape mutants

To locate the binding sites for mAbs LW9 and LW10, three escape mutants of the VR-299 strain were cloned and sequenced over the preVP2 coding region. A single nucleotide mismatch was found in each sequence (Table 2). All the substitutions occurred in the first or second position of the codon and modified the amino acid sequence. Interestingly, all the mutations were found in the two hypervariable regions previously identified. Substitutions of an arginine residue with tryptophan [in MLW9 (A1)] or with threonine [in MLW9 (P2)] were associated with a lower local hydrophilicity of the protein, compared to the profile observed in the corresponding region of Fig. 3. Arginine is a positively charged amino acid, while tryptophan is hydrophobic and threonine is polar but uncharged. The mutation observed in MLW10 (P4) is more conservative (glutamic acid to lysine) and had no significant influence on the hydrophilicity profile (not shown).

Two other escape mutants, obtained with mAbs LW9 and LW10, were cloned and sequenced. They were not included in Table 2 because they had the same nucleotide mismatch as that observed in MLW9 (P2) or in MLW10 (P4).

**DISCUSSION**

Comparison of aligned sequences of the preVP2 coding region revealed that IPNV strains can be classified in three distinct groups, as previously suggested (Heppell et al., 1993), though percentages of homology are not representative of the variations between entire A segments of the viral genome, because VP2 is more conserved than the rest of the polyprotein. Nevertheless, we identified two short hypervariable segments within the central region of the protein. The position and the size of the central variable domain are similar to those deter-

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### TABLE 1

#### Percentages of Homology between Deduced Amino Acid Sequences of the preVP2 Coding Region of Eight Different IPNV Strains

<table>
<thead>
<tr>
<th></th>
<th>VR-299</th>
<th>Jasper</th>
<th>DRT</th>
<th>Fr.21</th>
<th>N1</th>
<th>Ab</th>
<th>C2</th>
</tr>
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<tbody>
<tr>
<td>Jasper</td>
<td>100.0%</td>
<td></td>
<td>99.2</td>
<td>89.0</td>
<td>88.8</td>
<td>88.6</td>
<td>83.7</td>
</tr>
<tr>
<td>DRT</td>
<td>99.2</td>
<td>100.0%</td>
<td></td>
<td>89.0</td>
<td>88.8</td>
<td>88.6</td>
<td>83.7</td>
</tr>
<tr>
<td>Fr.21</td>
<td>89.0</td>
<td>89.0</td>
<td>100.0</td>
<td>89.6</td>
<td>88.4</td>
<td>88.4</td>
<td>83.9</td>
</tr>
<tr>
<td>N1</td>
<td>88.8</td>
<td>88.8</td>
<td>88.4</td>
<td>100.0</td>
<td>92.1</td>
<td>91.5</td>
<td>83.9</td>
</tr>
<tr>
<td>Ab</td>
<td>88.6</td>
<td>88.6</td>
<td>88.4</td>
<td>92.1</td>
<td>100.0</td>
<td>91.3</td>
<td>84.6</td>
</tr>
<tr>
<td>C2</td>
<td>88.8</td>
<td>88.8</td>
<td>89.4</td>
<td>91.5</td>
<td>91.3</td>
<td>100.0</td>
<td>85.8</td>
</tr>
<tr>
<td>He</td>
<td>83.7</td>
<td>83.7</td>
<td>83.9</td>
<td>88.4</td>
<td>88.2</td>
<td>84.6</td>
<td>85.8</td>
</tr>
</tbody>
</table>

* Sequence of the Jasper, DRT, and N1 strains were obtained from Duncan and Dobos (1986), Chung et al. (1993), and Håvarstein et al. (1990), respectively.

* Percentages were calculated with residues corresponding to positions 1 to 492 on the VR-299 sequence.
FIG. 4. Expression of truncated VP2s in bacteria and detection of specific proteins by Western blot. (A) Induced bacterial cell lysates run on a 10% SDS–PAGE and stained with Coomassie blue. Arrowheads show the positions of the fusion proteins. The molecular size markers (M) are indicated to the right. (B) Western blot of clone EMvar, which contains only the variable domain of VP2, probed with the anti-VR-299 serum and mAbs LW4, LW6, and LW7. (C to F) Western blots of truncated VP2s expressed in bacteria. The antibody used for the detection is indicated below each figure. A concentrated virus preparation (VR-299) was used as positive control. In (F), the position of native VP2/preVP2 and VP3 is indicated to the right, while arrowheads show the positions of specific bands corresponding to full-length and truncated VP2s in bacterial clones.

Amino acid substitutions in the escape mutants occurred in the two hypervariable segments. Thus, these regions are probably involved in the formation of the conformational epitope recognized by neutralizing mAbs LW9 and LW10. The locations of the substitutions observed in mutants MLW9 (A and P2) correlate with the discontinuous nature of the binding site for mAb LW9, as determined by Håvarstein et al. (1990), but the hypervariable segments are different since they found only one highly variable region (positions 234 to 264). These discrepancies are probably due to the fact that they compared only two strains, Jasper and N1.
TABLE 2

<table>
<thead>
<tr>
<th>Mutants (^a)</th>
<th>Nucleotide mismatches</th>
<th>Position</th>
<th>Amino acid mismatches</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLW9 (A)</td>
<td>C → T</td>
<td>882</td>
<td>R → W</td>
<td>255</td>
</tr>
<tr>
<td>MLW9 (P2)</td>
<td>G → C</td>
<td>976</td>
<td>R → T</td>
<td>286</td>
</tr>
<tr>
<td>MLW10 (P4)</td>
<td>G → A</td>
<td>864</td>
<td>E → K</td>
<td>249</td>
</tr>
</tbody>
</table>

\(^a\) Mutants MLW9 and MLW10 were selected with mAbs LW9 and LW10, respectively.

since they are probably too far apart (31 residues) to form a single continuous epitope. It is possible, however, that the antibodies bind to only one of the two hypervariable segments and that the other one plays a role in stabilizing the conformation of the epitope, as it was suggested for IBDV (Heine et al., 1991). Mutations observed in MLW9 (A and P2) are not conservative and have a significant local influence on the calculated hydrophilicity profile. Thus, they could slightly modify the folding of the protein.

Comparison of the different IPNV strains revealed significant differences in the hydrophilicity profile of the hypervariable region nearest the N-terminus (Fig. 3). Consequently, as suggested for the VR-299 escape mutants, both the primary sequence and the conformation of VP2 could contribute to the antigenic variations of IPNV. However, there is no direct evidence that the hypervariable regions of all IPNV strains contain an important neutralizing epitope.

Disulfide bonds are probably not important for the proper folding of the variable domain of VP2. In fact, only two conserved cysteines were found on all IPNV strains: one at position 55 and the other at position 195 (in the N-terminal part of the variable domain). Nevertheless, these residues could be involved in the formation of protein dimers and trimers since VP2 tends to polymerize in the absence of reducing agents (Dobos and Rowe, 1977). No other cysteines were found in the sequences, except for the one at position 202 on strains Fr.21 and C2.

Despite the fact that IPNV strains show more genomic and antigenic variations than IBDV, it appears that the antigenic structure is similar for both viruses. In fact, Azad et al. (1987) showed that the central region of IBDV VP2 (amino acids 206 to 350) contains a conformational epitope recognized by a neutralizing mAb. This region was found to be hydrophobic, with a small hydrophilic region located close to each terminus, which could correspond to the hypervariable regions we identified on IPNV. Most of the amino acid substitutions in IBDV also occur in the central portion of VP2 and especially in the two hydrophilic regions (Vakharia et al., 1994). Thus, these features could be shared by all birnaviruses, but their exact location and size are not identical.

In addition to the major conformational antigenic determinant, other important epitopes of IPNV are located on VP2. Tarrab et al. (1995) showed by additivity and competition assays that mAbs LW4, LW6, and LW7 (which recognize continuous epitopes) bind to proximal or identical sites. However, the relative position of these epitopes was uncertain. Results obtained with the truncated VP2s showed that LW4 binds to the region located between amino acids 331 and 351, which lies immediately downstream of the variable domain. The binding site for mAbs LW6 and LW7 could not be determined so precisely. Though they both reacted with the same bacterial clones, the intensity of the signal obtained with the truncated proteins was different for the two mAbs, while it was similar with the positive controls. Thus, LW6 and LW7 could bind to proximal but not identical epitopes, located between residues 271 and 331, in the variable domain. According to Tarrab et al. (1995), the binding site for LW6 would be closer to the LW4 epitope than to that of LW7.

To precisely determine the binding site for mAbs LW6 and LW7, escape mutants were isolated and sequenced as described previously for LW9 and LW10 mutants. However, because the neutralization titers of LW6 and LW7 were much lower than those of LW9 and LW10 (unpublished results), true stable mutants were difficult to obtain. Despite repeated attempts, no mismatches were detected in the VP2 coding region of the LW6 and LW7 escape mutants selected.

The reason why the antiserum seemed to react with the shorter clone EM245 and not with EM271 is unknown. It is possible that the truncated protein expressed in clone EM271 partially renatured itself during the transfer to the membrane in such a way that the epitopes were no longer accessible to the antibodies. Secondary folding into a more stable configuration has already been reported for proteins blotted from SDS gels (Nyholm and Ramlau, 1988). In clone EM271, one of the hypervariable segments is totally eliminated. Removal of this hydrophilic region leaves a hydrophobic terminus on the fusion protein, which could likely fold inside the protein or bind to the membrane. This could also explain why clone EM245, which does not contain this hydrophobic region, seemed to react with the antisera. Nevertheless, the weak signal obtained with EM245 would suggest that
minor continuous epitopes were located in the N-terminal part of the variable domain. However, the upstream conserved region did not seem to play an important role in the immunological response to the virus, although it has been suggested that, for the N1 strain, some of the amino acids located between positions 153 and 203 could be involved in the formation of a conformational epitope (Frost et al., 1995).

If refolding of the fusion protein in clone EM271 hindered the binding of the antibodies to the epitopes close to its C-terminus, then it is likely that the negative results obtained for mAbs LW6 and LW7 with this bacterial clone resulted from the same situation. In such a case, it is impossible to determine if the binding site for these two mAbs lies between residues 271 and 331, as mentioned before, or between residues 245 and 271. Refolding of IPNV VP2 after blotting onto a membrane has been suggested previously (Frost et al., 1995; Liao and Dobos, 1995), except that in these particular cases it was thought to restore a discontinuous epitope.

There is no direct evidence that anti-IPNV antibodies with an in vitro neutralizing activity are involved in the protection of fish. However, experiments conducted with another trout virus, the Egtved virus, showed that in vitro neutralizing, as well as nonneutralizing, mAbs have an in vivo protective ability (Lorenzen et al., 1990). Passively transferred antisera from trout or mammals into fish also provided protection against different trout pathogens (Harrell et al., 1975; LaPatra et al., 1994). Accordingly, an antigen which contains important epitopes of the virus is likely to induce a protective immune response in fish.

Results reported herein suggest that a recombinant subunit vaccine against IPNV should include at least the variable domain of VP2 and the 20 adjacent amino acids of the conserved C-terminal part (residues 183 to 351). The efficiency of immunization of trout with such vaccines has not been tested thoroughly, but some experiments showed that vaccination with the entire polyprotein (VP2, NS, and VP3) induced a good protection in rainbow trout fry (Manning and Leong, 1990), while VP2 alone (or its amino-terminus) is less effective (Bootland et al., 1993). In both cases, however, proteins were expressed in bacteria. It is possible that VP2 does not fold properly in prokaryotic cells. Thus, fish immunized with such proteins could not raise antibodies to the important conformational epitope of the variable domain. In this article, it has not been possible to detect VP2 produced in E. coli with mAbs LW9 and LW10, even when bacteria were lysed in nondenaturating conditions.

In addition to the proper folding of the protein, glycosylation could also play an important role in antigenicity (Caust et al., 1987). It has already been shown that IPNV VP2 contains carbohydrate residues that are probably N-linked (Estay et al., 1990). Comparison of sequences revealed that few potential N-glycosylation sites are found on the protein, three of which are conserved among all strains: two in the N-terminal conserved region (positions 73 and 118) and one in the variable domain (position 234). Consequently, VP2 expressed in eukaryotic cells could be more effective at inducing a good protective immune response when injected into fish, as is the case for IBDV in chickens (Jagadish et al., 1990; Azad et al., 1991).

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