The Nucleotide Sequence of Human Rhinovirus 1B: Molecular Relationships within the Rhinovirus Genus

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SUMMARY

We have determined the complete nucleotide sequence of human rhinovirus 1B and made comparisons with other rhinoviruses. Extensive homology was found with serotypes 2 and 89 but the similarity to serotype 14 was considerably less. Rhinovirus-specific characteristics have been noted, in particular the length of the 5' non-coding region and the pattern of codon usage, and these may be sufficient to define the rhinoviruses as a distinct genus rather than being considered as members of the enteroviruses as has been suggested previously.

INTRODUCTION

Human rhinoviruses (HRVs) are the major causative agents of the common cold, a disease of considerable economic importance and one of the most frequent virus infections of man (Fox et al., 1985). Major factors in the incidence of the disease are the existence of over 100 immunologically distinct HRV serotypes and the fact that several of these can co-circulate within the community (Gwaltney, 1975). This serotype diversity probably precludes a vaccination programme based on conventional methodologies and necessitates the development of alternative forms of control, a process which would be facilitated by a better understanding of the viruses.

Rhinoviruses, including HRVs, form the largest genus of the family Picornaviridae. They are composed of an icosahedral capsid made up of 60 copies of each of four virus-coded proteins (VP1 to VP4) enclosing a single-stranded RNA genome of about 7200 nucleotides (Stanway et al., 1984a; Callahan et al., 1985; Skern et al., 1985; Duechler et al., 1987; Rossmann et al., 1985). As in other picornaviruses, the RNA is of positive polarity, is polyadenylated at its 3' terminus and has a small protein, VPg, covalently attached to the 5' terminus (Ahlquist & Kaesberg, 1979). The RNA encodes one long polyprotein which is cleaved by virus proteases to give the mature proteins. This long open reading frame is preceded by a 5' non-coding region, approximately 600 nucleotides in length and of unknown function.

The complete nucleotide sequences of the genomes of three rhinoviruses, HRV-14, HRV-2 and more recently HRV-89 have been determined (Stanway et al., 1984a; Callahan et al., 1985; Skern et al., 1985; Duechler et al., 1987). The availability of further HRV nucleotide sequences is important for several reasons. Firstly, comparisons between HRV-14 and HRV-2 showed a relatively low degree of homology, indeed a similar level to that seen between either virus and the polioviruses, members of the enterovirus genus of the family Picornaviridae (Skern et al., 1985). This indicated that the rhinovirus genus is relatively diverse. When the sequence of HRV-89 was determined it was found that this serotype is highly homologous to HRV-2 (Duechler et al., 1987). Further sequencing is necessary to assess properly the level of serotype diversity and to find out whether there are groups of more closely related viruses within the genus. This could have important implications for the design of synthetic, broadly reactive vaccines. Secondly,
previous results have demonstrated a great deal of sequence conservation within the 5' non-coding region, indicating that this part of the genome has a vital function (Stanway et al., 1984a; Skern et al., 1985). The availability of other nucleotide sequences would facilitate the identification of important sequences within this region. Finally, rhinoviruses can be grouped according to which cellular receptor they recognize. About 85% (including HRV-14 and HRV-89) recognize one receptor and a minor group (including HRV-2) utilize a second (Abraham & Colonno, 1984; Colonno et al., 1986). Sequence comparisons within and between receptor groupings may give information on the molecular basis of receptor specificity. This is particularly true in view of the availability of the three-dimensional structure of HRV-14 and the suggestion that a striking surface feature, a deep ‘canyon’, may be involved in receptor binding (Rossmann et al., 1985).

Here we present the complete nucleotide sequence of HRV-1B, a member of the minor receptor group, and discuss our results in terms of molecular relationships within the rhinovirus genus, our understanding of the 5' non-coding region and the basis of receptor specificity.

METHODS

Virus. HRV-1B, obtained from the MRC Common Cold Unit, Salisbury, U.K., was propagated and purified as already described (Stanway et al., 1984a).

Molecular cloning. The purification of RNA from HRV-1B and the cloning of cDNA-RNA hybrids was as described previously except that different reverse transcription conditions were used and the vector was dG-tailed pBR322 (Stanway et al., 1984b). Reverse transcription took place for 1 h at 37 °C in a 50 µl reaction volume containing 50 mM-Tris–HCl pH 7.5, 75 mM-KCl, 10 mM-dithiothreitol, 3 mM-MgCl₂, 0.5 mM of each dNTP, 10 µg/ml oligo(dT), 100 µg/ml bovine serum albumin and 200 units of Moloney murine leukaemia virus (MMLV) reverse transcriptase (Bethesda Research Laboratories).

Approximately 350 tetracycline-resistant, ampicillin-sensitive transformants were obtained and these were screened initially by hybridization using an oligo(dT)-primed cDNA probe made with limiting concentrations of nucleotides to detect the presence of HRV-1B sequences proximal to the 3' terminus (Cann et al., 1983). Of the cDNA inserts identified the one representing the 5'-most sequences (pOB512) was then used to probe for 5' proximal sequences. These two probing experiments enabled overlapping cDNA clones representing the complete genome to be identified.

One cDNA insert, pOB512, was only 200 bp short of full-length and several were over 4 kb in size. This contrasts with our previous experience with another rhinovirus, HRV-14, for which cDNA inserts did not exceed 3 kb (Stanway et al., 1984a). We attribute this improvement to the use of MMLV reverse transcriptase in the present experiment rather than the avian myeloblastosis virus enzyme used formerly. We have also obtained large cDNA clones during the molecular cloning of other rhinovirus genomes using MMLV reverse transcriptase.

DNA sequencing. HRV-1B cDNA contains four internal PstI sites. For sequencing, the largest of the PstI fragments (2.1 and 3.8 kb) were subcloned into M 13mp 19 in both orientations and a series of nested deletions were produced by the method of Dale et al. (1985). The smaller PstI fragments were digested further with Sau3A and/or HaeII and subcloned into M13. Nucleotide sequences were determined by the dideoxynucleotide method and data were collated and analysed using published computer programs (Staden, 1980, 1982).

RESULTS AND DISCUSSION

Comparison with other rhinoviruses

The complete nucleotide sequence of HRV-1B derived from cloned cDNA is shown in Fig. 1. The sequence of 7133 nucleotides plus a poly(A) tail is similar in length to the other rhinoviruses studied and has a similar nucleotide composition (A, 34.1%; C, 17.4%; G, 19.5%; T, 29.0%) being somewhat A + T-rich. A comparison of the predicted amino acid sequence of the virus proteins with those of HRV-2, HRV-14 and HRV-89 (Stanway et al., 1984a; Callahan et al., 1985; Skern et al., 1985; Duechler et al., 1987), the other three sequenced rhinoviruses, is shown in Table 1. All the proteins of HRV-1B show considerable homology with those of the other rhinoviruses, but the relationship to HRV-2 and to HRV-89 is much more marked than that to HRV-14. The greatest degree of similarity is to HRV-2 where the homology within the individual proteins does not fall below 74% and in many of the proteins there is a much higher degree of conservation. The relationship to HRV-89 is somewhat less throughout much of the genome except that in VP4, VP2, VP3 and P3-A there is marginally greater homology than to
Human rhinovirus 1B

Table 1. Protein homologies (%) between rhinoviruses

<table>
<thead>
<tr>
<th>Protein</th>
<th>HRV-1B/HRV-2</th>
<th>HRV-1B/HRV-89</th>
<th>HRV-1B/HRV-14</th>
<th>HRV-2/HRV-89</th>
<th>HRV-2/HRV-14</th>
<th>HRV-14/HRV-89</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP4</td>
<td>94</td>
<td>100</td>
<td>52</td>
<td>94</td>
<td>51</td>
<td>54</td>
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<td>VP2</td>
<td>77</td>
<td>78</td>
<td>61</td>
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<td>VP3</td>
<td>74</td>
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<td>51</td>
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<td>VP1</td>
<td>74</td>
<td>67</td>
<td>36</td>
<td>62</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>P2-A</td>
<td>88</td>
<td>83</td>
<td>43</td>
<td>85</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>P2-B</td>
<td>88</td>
<td>68</td>
<td>39</td>
<td>64</td>
<td>42</td>
<td>45</td>
</tr>
<tr>
<td>P2-C</td>
<td>84</td>
<td>74</td>
<td>50</td>
<td>72</td>
<td>44</td>
<td>49</td>
</tr>
<tr>
<td>P3-A</td>
<td>74</td>
<td>75</td>
<td>52</td>
<td>65</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>VPg</td>
<td>91</td>
<td>81</td>
<td>57</td>
<td>86</td>
<td>58</td>
<td>57</td>
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<td>Protease</td>
<td>83</td>
<td>76</td>
<td>52</td>
<td>75</td>
<td>50</td>
<td>52</td>
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<tr>
<td>Polymerase</td>
<td>83</td>
<td>72</td>
<td>55</td>
<td>72</td>
<td>55</td>
<td>58</td>
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</tbody>
</table>

HRV-2. HRV-1B and HRV-2 are the most closely related serotypes overall since their degree of homology also exceeds that seen in the HRV-2/HRV-89 comparison. In contrast to the relationship to serotypes 2 and 89, the homology to HRV-14 is of a much lower level, varying from 36% to 61%. These are similar figures to the HRV-2/HRV-14 and HRV-89/HRV-14 comparisons although overall HRV-14 seems to be less similar to HRV-2 than to the other two serotypes.

The distribution of amino acid differences between HRV-1B and each of the three previously sequenced rhinoviruses is shown in Fig. 2. The polyprotein has been divided into blocks of four amino acids and a vertical bar plotted of different height depending on whether none, one, two, three or four of the amino acids in each block are different. Interestingly the degree of conservation seems to be remarkably uniform throughout the genome and this is slightly different from the pattern seen with other closely related picornaviruses which show more homology in the non-structural proteins. Within the capsid region the homologies between the three closely related rhinoviruses are in the range 62 to 100% and for HRV-1B/HRV-2 are 74 to 94% (Table 1). These figures approach those for the corresponding homologies between poliovirus serotypes (71 to 92% for PV-1/PV-3) (Stanway et al., 1983). In comparison to the poliovirus situation, the homologies of some of the proteins towards the C-terminal end of the polyprotein, particularly the polymerase (72 to 83%) seem relatively low. In the polioviruses this protein is almost identical between serotypes (about 98% homology) and is far more homologous than the capsid proteins except for VP4 (Toyoda et al., 1984). This pattern of conservation of the polymerase gene and a more flexible structure for the capsid proteins does not seem to be as marked between HRV-1B, -2 and -89. It is therefore possible that the rhinovirus polymerase is less stringent in its requirement for absolute maintenance of structure than its poliovirus counterpart. On the basis of partial sequence comparisons between HRV-14 and HRV-2, we suggested previously that the rhinoviruses are a very diverse group (Stanway et al., 1984a). The current results on HRV-1B as well as the HRV-89 sequence indicate that this conclusion must be modified. Clearly there is considerable diversity between some serotypes, e.g. between HRV-14 and each of HRV-2, -1B and -89, but there are also groups of more closely related serotypes. Since HRV-1B is in the same receptor group as HRV-2 (the minor group) it would be expected that it would share more overall homology with this serotype than with HRV-14, a member of the major group. However, the high level of homology of HRV-1B and HRV-2 to HRV-89 (also a member of the major group) shows that receptor grouping cannot account for overall genetic homology. In support of this conclusion we have sequence data indicating that HRV-1B is closely related to several other rhinoviruses in the major receptor group. The detailed picture of the relationships within the genus will become clear with the availability of further complete or partial sequences.
Fig. 1. The nucleotide and predicted amino acid sequences of cDNA representing the entire genome of HRV-1B. Protein boundaries were predicted by alignment with other rhinoviruses.
Fig. 2. The amino acid homologies of HRV-1B to HRVs 2, 89 and 14. The genome has been divided into blocks of four amino acids and a vertical line of height 0 to 4 plotted to represent the number of amino acid mismatches within the block.

Table 2. Nucleotide frequencies in rhinoviruses and enteroviruses

<table>
<thead>
<tr>
<th>Position in codon</th>
<th>HRV-1B</th>
<th>HRV-2</th>
<th>HRB-89</th>
<th>HRV-14</th>
<th>PV-3*</th>
<th>CB-4†</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>20.5</td>
<td>20.5</td>
<td>21.1</td>
<td>19.9</td>
<td>19.3</td>
<td>19.9</td>
</tr>
<tr>
<td>A</td>
<td>35.0</td>
<td>35.2</td>
<td>31.9</td>
<td>33.6</td>
<td>31.8</td>
<td>30.7</td>
</tr>
<tr>
<td>C</td>
<td>16.3</td>
<td>16.6</td>
<td>17.6</td>
<td>17.7</td>
<td>18.0</td>
<td>17.9</td>
</tr>
<tr>
<td>G</td>
<td>28.2</td>
<td>27.7</td>
<td>29.4</td>
<td>28.8</td>
<td>30.9</td>
<td>31.5</td>
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<td>Second</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>U</td>
<td>28.2</td>
<td>28.2</td>
<td>28.6</td>
<td>28.9</td>
<td>27.4</td>
<td>28.3</td>
</tr>
<tr>
<td>A</td>
<td>32.9</td>
<td>33.0</td>
<td>31.2</td>
<td>31.9</td>
<td>31.8</td>
<td>31.5</td>
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<tr>
<td>C</td>
<td>22.5</td>
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<td>23.7</td>
<td>23.8</td>
<td>24.9</td>
<td>22.9</td>
</tr>
<tr>
<td>G</td>
<td>16.4</td>
<td>16.3</td>
<td>16.5</td>
<td>15.4</td>
<td>15.9</td>
<td>17.3</td>
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<tr>
<td>Third</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>38.6</td>
<td>36.0</td>
<td>39.0</td>
<td>32.4</td>
<td>25.2</td>
<td>22.8</td>
</tr>
<tr>
<td>A</td>
<td>34.7</td>
<td>32.8</td>
<td>33.4</td>
<td>33.6</td>
<td>25.8</td>
<td>24.4</td>
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<tr>
<td>C</td>
<td>13.7</td>
<td>15.9</td>
<td>15.2</td>
<td>17.8</td>
<td>26.2</td>
<td>27.2</td>
</tr>
<tr>
<td>G</td>
<td>13.0</td>
<td>15.3</td>
<td>12.4</td>
<td>16.2</td>
<td>22.8</td>
<td>25.6</td>
</tr>
</tbody>
</table>

* Poliovirus type 3 (Stanway et al., 1983).
† Coxsackievirus B4 (Jenkins et al., 1987).

Comparisons with viruses sequenced previously enable the prediction of the cleavage sites in the polyprotein which give rise to the mature virus proteins. These have been well established for the polioviruses and the capsid cleavage sites have been determined for HRV-2 and HRV-89 by protein sequencing and for HRV-14 from the three-dimensional structure (Kitamura et al., 1981; Skern et al., 1985; Duechler et al., 1987; Rossmann et al., 1985). In all these viruses the sequence QG predominates as the major recognition site for the virus protease protein 3C, which performs most of the cleavages. At all junctions in HRV-1B, predicted by alignment with
the sequences of other viruses, there is a corresponding QG except for the VP3/VP1 (QN as in HRV-2) and P2-B/P2-C (probably ES) boundaries. Thus QG is probably the preferred cleavage site for the HRV-1B 3C protease. The site for the second protease, P2-A, is less clear due to poor homology between the rhinoviruses in the region of the boundary (VP1/P2-A). Assuming that the HRV-1B VP1 protein is of similar length to that of HRV-14 (289 amino acids) this cleavage should occur somewhere around position 3380 in the primary structure. The sequence YG, the recognition sequence for the P2-A protease of the polioviruses and HRV-14, is absent from this region and thus a different signal must be utilized. We suggest that the VG sequence marked on Fig. 1 is used but this needs to be confirmed by direct protein sequence analysis.

Recently, the nucleotide sequence of the P3-C protease region of HRV-1A has been published and this gives the possibility of a preliminary comparison (Werner et al., 1986). The two viruses are remarkably homologous in this region, 89.6% and 96.7% at the nucleotide and amino acid levels respectively. This is much greater homology than that seen between any other pair of rhinoviruses and it may suggest that HRV-1A and HRV-1B are correctly regarded as subtypes rather than distinct serotypes.

**Comparison with enteroviruses**

Our previous results on HRV-14 indicated that the rhinovirus and enterovirus genera are closely related; indeed, throughout much of the genome, HRV-14 was found to be more homologous to the polioviruses (enteroviruses) than to HRV-2 (Stanway et al., 1984a; Skern et al., 1985). This prompted us to suggest that rhinoviruses and enteroviruses can be considered as one genus of the family Picornaviridae rather than being divided as at present (Stanway et al., 1984a). The present finding of tighter grouping within the rhinovirus genus tends to weaken our argument about the amalgamation of the enteroviruses and rhinoviruses. It is also interesting that as more information becomes available, distinct rhinovirus characteristics are emerging. For instance, if codon usage is analysed, it can be seen that in the first two positions of the triplet, the occurrence of each nucleotide follows more or less the same pattern between the enteroviruses and rhinoviruses (Table 2). However, there is a marked difference in the third base position, where A, G, C and T occur about equally in the enteroviruses but where there is a preponderance of A + T in the rhinoviruses. This effect is particularly striking in HRV-1B and HRV-89 where 73.3% and 72.4% respectively of the codons have an A or T in the third position. Considering only the codons where the amino acid is specified by the first two nucleotides (i.e. any nucleotide can be in the third position without changing the amino acid) the figure for A + T in this position rises to over 80%. We currently believe that this A and T preference is a result of the low optimum temperature for growth of rhinoviruses (33 °C) and that it may be related to the weakness of A-T base pairs compared with G-C. Base pairing is marginally more stable at 33 °C than at the optimum temperature for enteroviruses (37 °C) and if this adversely affects some stage of viral replication a drift to a higher A + T content would be advantageous. It may be envisaged that this could be important during at least two stages of the replicative cycle. Firstly, during RNA replication weaker interactions between the nascent and template strands may facilitate strand displacement and lead to enhanced replication. Secondly, during translation of the long open reading frame the high A + T content could lead to a reduction of fortuitous secondary structure which may otherwise inhibit translation.

**The non-coding regions**

As with other picornaviruses, the 5' non-coding region is one of the most interesting regions of the HRV-1B genome. This region is highly conserved between related viruses and is the most homologous part of the genome when rhinoviruses and enteroviruses are compared (Stanway et al., 1984a; Skern et al., 1985). One important observation which has implications for classification is the fact that this region is of very similar length (about 620 nucleotides) in the three rhinoviruses sequenced to date but is much shorter than that of the enteroviruses (about 740 nucleotides; Toyoda et al., 1984; Jenkins et al., 1987). This difference in length is due to a relative deletion or insertion immediately prior to the AUG that initiates the long open reading frame. The HRV-1B 5' non-coding region is 622 nucleotides in length, a length similar to that of
the other rhinoviruses. This suggests that the length of this region of the genome may be approximately the same in all rhinoviruses but it is not clear how this could contribute to the characteristic properties of the genus.

A further interesting feature is that prior to the initiation codon there is a characteristic A + T-rich region in HRV-14, HRV-2 and HRV-89 which although not exactly conserved between the serotypes, nevertheless shows great similarity (Fig. 3). At the corresponding point of HRV-1B there is a sequence very similar to that found in HRV-2 and such a sequence is also found in HRV-85 (unpublished observations). In view of its location this sequence may play some role in initiation of translation of the rhinovirus long open reading frame.

An analysis of the relationship between HRV-1B and HRV-2, HRV-14 and HRV-89 throughout the 5' non-coding region demonstrates that in this region, as in the rest of the genome, HRV-1B is most closely related to HRV-2 and to HRV-89. The overall homology is 83.1% and 80.5% respectively. Homology is found throughout the 5' non-coding region but certain blocks of conservation are particularly evident. Although the overall homology to HRV-14 is less (69.1%) there is striking conservation of two of these blocks (found around positions 180 and 440 to 560). These sequences, which are also seen in the enteroviruses, must play some central role in the virus replicative cycle but this role, and indeed the function of the whole region, has not yet been defined. Interestingly, in poliovirus type 3 a single mutation in the vicinity of one of the blocks (position 472) has been shown to exert a marked effect on the virulence of different strains (Evans et al., 1985). Most of the differences between the four rhinovirus serotypes in the 5' non-coding region are base substitutions but at position 98 in HRV-1B there is some degree of length variability since HRV-14 has an insertion of 13 to 17 nucleotides relative to the other three serotypes. This again suggests that HRV-14 is more distantly related to the other serotypes.

In confirmation of the importance of the non-coding regions, the 3' non-coding region of HRV-1B also exhibits rhinovirus-specific characteristics. Whereas in enteroviruses the region is about 70 to 120 nucleotides long, the four sequenced rhinoviruses have much shorter non-coding regions, about 40 nucleotides long. There is also some nucleotide homology between all the rhinoviruses and this is considerable between HRV-IB, HRV-2 and HRV-89. For instance the last 17 nucleotides before the poly(A) tract are identical between HRV-2 and HRV-89 and there is a stretch of 13 identical nucleotides towards the start of the 3' non-coding region between HRV-1B and HRV-2. There is essentially no homology with the enteroviruses.

The virion surface

The homology to previously sequenced viruses enables the location of features such as sites involved in virus neutralization. For HRV-14 several such sites have been identified and termed NIm-IA, NIm-IB, NIm-II and NIm-III (Sherry et al., 1986). When HRV-1B is compared with the other rhinoviruses it is found that these regions are highly diverse (Fig. 2). This is true even when the comparison is with HRV-2 and HRV-89 where there is otherwise a high degree of homology. By aligning the sequence with that of HRV-14 it is clear that the diverse regions are in the exterior loops of the capsid proteins and outside the β-barrel core structures (data not shown). Most of the diversity is due to amino acid substitution but there are also several small deletions and insertions. In view of these characteristics it is likely that these areas of HRV-1B are recognized by neutralizing antibodies.
Another interesting aspect of the capsid sequence is the conservation of the N terminus of VP1 between HRV-1B, HRV-2 and HRV-89. This part of VP1 has not been shown to induce neutralizing antibodies, indeed it is believed to be buried within the capsid. However, between the three serotypes of poliovirus there is considerable diversity and such diversity is also seen between HRV-14 and HRV-2. In contrast only one amino acid of the first 20 is different between HRV-1B, HRV-2 and HRV-89. The reason for this pattern of homology is not clear.

The determination of the three-dimensional structures of HRV-14 and poliovirus type 1 (Rossmann et al., 1985; Hogle et al., 1985) have important implications for an understanding of virus assembly, uncoating and interaction with the host cell, which are attractive targets for novel anti-rhinoviral agents. One obvious target is the initial interaction between the cell receptor and the receptor-binding site on the virus surface. The identification of the receptor-binding domain could lead to the development of agents which block this interaction and thus act therapeutically against rhinovirus infections. Deep 'canyon' structures on the surface of HRV-14 have been postulated as the receptor-binding sites of this virus (Rossmann et al., 1985; Smith et al., 1986). It might be expected that members of the same receptor group would show some similarity of primary structure in the areas which make up the receptor-binding domain. We have compared the predicted amino acid sequences of HRV-1B, HRV-2, HRV-14 and HRV-89 in the regions of the genome which make up the 'canyon' but the results are somewhat equivocal (data not shown). Even between HRV-1B and HRV-2, members of the same group, there are considerable differences in these regions, although these are not as great as when each serotype is compared with HRV-14. This may be just a reflection of the close overall similarity between HRV-1B and HRV-2. An exception is the C terminus of VP3, which partially lines the 'canyon'. Here HRV-1B and HRV-2 are closely related and are very diverse from HRV-14. However, the homology to HRV-89 is also quite high in this region of VP3. The definitive assessment of the role of this surface feature in receptor binding, however, must await further sequence comparisons and the direct manipulation of the region via an infectious cDNA copy of the genome. These experiments are currently in progress.

In conclusion, the complete nucleotide sequence of HRV-1B presented here is important for several reasons. It gives a further indication of the relationship between rhinovirus serotypes and confirms that some share more extensive homology than previously thought. It emphasizes the importance of the 5' non-coding region and it strengthens the conclusion that there are certain rhinovirus-specific features such as the length of the 5' and 3' non-coding regions and codon usage. We are currently exploring the role of the 5' non-coding region and whether these rhinovirus-specific features determine the characteristic properties of the viruses.

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REFERENCES


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