Rhinoviral receptor discrimination: mutational changes in the canyon regions of human rhinovirus types 2 and 14 indicate a different site of interaction

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Amino acid sequence comparisons between the capsid proteins of several human rhinovirus (HRV) serotypes identified residues potentially involved in the discrimination between the major and the minor group receptors. Amino acids conserved within minor group HRVs were substituted in a full-length cDNA clone of HRV2 for those found at equivalent positions in major group HRVs. Transfection of HeLa cells with RNAs transcribed from seven individual mutated cDNAs gave rise to only two viable viruses; growth characteristics and affinity for the minor group receptor of both were unchanged compared to wild-type. Similar mutations in HRV14 were previously shown to alter the affinity for its receptor; the contact sites between the minor group viruses and the respective receptor may therefore be different.

With one exception, human rhinoviruses (HRVs) utilize one of two receptors to gain entry into the cell; the majority, referred to as the major group, bind to the protein known as ICAM-1 (intercellular adhesion molecule-1; Abraham & Colonno, 1984; Uncapher et al., 1991) whereas the minor group (about 10% of HRVs) use a protein of about 120K with unknown function (Hofer et al., 1992). Characteristic surface depressions of the capsids of these small icosahedral RNA-containing viruses are thought to be involved in binding to the receptors. These depressions in the viral capsid (referred to as the ‘canyon’) encircling the fivefold axis of symmetry are only about 3 nm wide; this has led to the hypothesis that amino acid residues in this region are inaccessible to immunoglobulins and therefore are not subject to immunological pressure (Rossmann et al., 1985). The various rhinovirus serotypes belonging to one receptor group could thus conserve structures important for the interaction with their respective receptors whilst permitting serotypic diversity (Rossmann, 1989). Furthermore, the hypothesis implies that differences in canyon structure between major and minor group rhinoviruses must be responsible for specific recognition of the two different receptors (‘receptor discrimination’).

In its simplest form, this would involve a set of amino acid residues conserved at certain positions in major group viruses and a second set common only to minor group viruses.

Fig. 1 shows part of a sequence comparison designed to locate potential discriminatory sites. Initial searches showed four possible regions in or on the rim of the canyon, conserved in minor group viruses, but not in major group ones. These were basic residues at positions 1081 (HRV2 numbering; see Skern et al., 1985; Blaas et al., 1987) whereas the minor group (about 10% of HRVs) use a protein of about 120K with unknown function (Hofer et al., 1992). Characteristic surface depressions of the capsids of these small icosahedral RNA-containing viruses are thought to be involved in binding to the receptors. These depressions in the viral capsid (referred to as the ‘canyon’) encircling the fivefold axis of symmetry are only about 3 nm wide; this has led to the hypothesis that amino acid residues in this region are inaccessible to immunoglobulins and therefore are not subject to immunological pressure (Rossmann et al., 1985). The various rhinovirus serotypes belonging to one receptor group could thus conserve structures important for the interaction with their respective receptors whilst permitting serotypic diversity (Rossmann, 1989). Furthermore, the hypothesis implies that differences in canyon structure between major and minor group rhinoviruses must be responsible for specific recognition of the two different receptors (‘receptor discrimination’).

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The transfected HeLa cells were incubated for 3 days at 34 °C, and the virus titre in the supernatant was measured by plaque tests. Transfection with RNAs transcribed from the wild-type pHRV2/1, mutant HRV2_{2146P-g} and HRV2_{3185R-T} all gave a yield of about 300 p.f.u./ml. No differences in plaque size and morphology between the wild-type and these mutants were observed. In contrast, no viable virus was recovered upon transfection with RNA carrying the single amino acid changes 1081K:E and 3229L:T or the tripeptide sequence derived from HRV14, HRV39 or HRV89 used to replace the TEK sequence at position 1222.

From the two viable mutants, virus stock was obtained from a single plaque, the RNA was isolated and reverse-transcribed. The mutagenized regions were amplified by PCR and the presence of the respective mutations was verified by direct DNA sequencing.

Virus was labelled in vivo with \[^{35}S\]methionine and purified as described (Neubauer et al., 1987); the binding to HeLa cells as a function of the incubation time was then determined for wild-type HRV2, HRV2_{1148P-g} and HRV2_{3185R-T}. As can be seen in Fig. 2, no significant difference in binding between wild-type HRV2 and the mutants was apparent. This is in contrast to binding experiments carried out by Colonno et al. (1989) under similar conditions which showed that twice as much of the corresponding HRV14_{1155P-g} mutant was bound than wild-type HRV14.

In order to check the receptor specificity, competition experiments were carried out to assess whether the mutants still bind to the minor group rhinovirus receptor. When increasing amounts of unlabelled HRV were present during the incubation of HeLa cells with \[^{35}S\]methionine-labelled mutant virus, binding was reduced only by HRV2 whereas the same amount of HRV2msma and HRV2msa were eluted from the cells. As can be seen in Fig. 2, no significant difference in binding between wild-type HRV2 and the mutants was apparent. This is in contrast to binding experiments carried out by Colonno et al. (1989) under similar conditions which showed that twice as much of the corresponding HRV14_{1155P-g} mutant was bound than wild-type HRV14.

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Fig. 2. Binding kinetics of HRV2<sub>1148P:G</sub> and HRV2<sub>3182R:T</sub> compared to wild-type HRV2. Two independent experiments are shown; binding is given as a percentage of total counts attached to the cells. The binding assays were carried out as described by Colonno et al. (1988) except that cells were used instead of crude cell membrane preparations. HeLa cells ($3 \times 10^6$) from suspension culture (Wisconsin strain, obtained from R. Rueckert) in 2 ml infection medium (Neubauer et al., 1987) were incubated at room temperature under slow agitation with 20000 c.p.m. of $^{35}$S-labelled wild-type HRV2 (○), HRV2<sub>1148P:G</sub> (▲) or HRV2<sub>3182R:T</sub> (●). At the times indicated, 400 µl samples were withdrawn, the cells were pelleted and the radioactivity in the supernatants and the pellets was determined by liquid scintillation counting. Non-specific binding as determined by use of virus heated to 56 °C (Lonberg-Holm & Yin, 1973) was less than 10% (data not shown).

Fig. 3. Competition of unlabelled HRV2 or HRV14 for the binding of viral mutants to HeLa cell membranes. Crude membranes corresponding to about $1 \times 10^7$ HeLa cells (Hofer et al., 1992) were incubated with 20000 c.p.m. of $^{35}$S-labelled HRV2<sub>1148P:G</sub> or HRV2<sub>3182R:T</sub> in 500 µl infection medium at room temperature for 45 min in the presence of the indicated number of p.f.u. of unlabelled HRV2 (○) or HRV14 (■), respectively. The membranes were pelleted in an Eppendorf centrifuge at 14000 r.p.m. for 10 min and the radioactivity in the pellets and the supernatants was determined by scintillation counting. The percentage of total counts found in the pellets is indicated.

Why this amino acid is conserved at the equivalent position in all picornaviruses remains unknown; possibly mechanisms not operable under tissue culture conditions confer a selective growth advantage to viruses with Pro at this position. Similarly, the presence of Arg at position 182 in VP3 of HRV2 is not required for binding to HeLa...
cells, since the presence of Thr does not alter either the affinity for the receptor or the growth characteristics. When the sequence of HRV9, a major group virus, became available (G. Leckie & J. W. Almond, personal communication), the latter result could be explained as HRV9 also possesses a Lys at the equivalent position. However, whereas basic, hydrophilic and acidic residues are tolerated by major group viruses at the position corresponding to 1081 in HRV2, Glu was not acceptable for HRV2. No conserved oligopeptide sequence corresponding to the TEK element was evident for major group viruses.

The reason why the mutations HRV21081K; E-; HRV22290L; T and the ‘TEK’ mutations are not viable is currently not understood; unfortunately, the three-dimensional structure of HRV2 has not yet been determined. However, the structure of HRV1A, which is closely related to HRV2 in the amino acid sequence of the capsid proteins, is known (Kim et al., 1989). As HRV1A also belongs to the minor receptor group, its structure was used as a model to predict the positions of the individual amino acids in the capsid of HRV2. Based on this, no indication for steric or electrostatic interference of the amino acid side-chains changed in the mutants was found; moreover, all residues are expected to be on the surface and exposed to solvent.

The transfection efficiency of HRV2 RNA is low; transfection with HRV2 RNA gives only 50 plaques/μg, with either viral or in vitro transcribed RNA. In contrast, 10^6 plaques/μg HRV14 RNA can be achieved (Duechler et al., 1989; Skern et al., 1991). Reasons for this difference are not clear. Thus it was not possible to examine in vivo 35S-labelled viral proteins produced in cells transfected with mutant RNAs. Therefore, we attempted to isolate viral particles from the transfected cells by immunoprecipitation and to amplify virus-specific DNA by reverse transcription and PCR technology. However, amplification of virus-specific DNA remaining from the transfection could never be excluded rendering the results inconclusive. At the present time therefore we cannot exclude that the HRV21081K; E-; HRV22290L; T and ‘TEK’ mutations are interfering with steps in the viral life cycle other than attachment to the receptor.

The change of Pro1148 to Gly in HRV2 was without any effect on the affinity of the virus for its cell surface receptor. In HRV14, the equivalent change led to a considerably tighter binding to its corresponding receptor; Pro1155 forms a shelf near the bottom of the canyon and seems to prevent the receptor from moving deeper into the viral capsid. The increase in binding affinity upon replacement of this Pro by Gly was therefore interpreted as a reduction of steric hindrance (Giranda et al., 1990). Since no such effect was seen in HRV2, it is likely that the mode of interaction of HRV minor group serotypes with their receptor is different from those of major group viruses.

Although the outline of the canyon in minor and major group HRVs and poliovirus is quite similar, the fine structure differs considerably. As ICAM-1 and the poliovirus receptor both belong to the immunoglobulin superfamily, it was assumed that the minor group receptor would share structural features with this family and that its interactions with the viral capsid would be similar. However, whether the minor group receptor is also related to this group of proteins remains to be determined. For these reasons it is possible that the attachment site of minor group virions differs considerably from the one identified for major group HRVs (Olson et al., 1993).

We wish to thank Herbert Auer, Martin Grünberger and Karin Stasny for their help and Z. Rattler for invaluable discussions. This work was supported by the ‘Österreichischer Fonds zur Förderung der Wissenschaftlichen Forschung’, the Austrian Ministry of Science, and by Boehringer Ingelheim.

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(Received 14 October 1992; Accepted 17 May 1993)