Human rhinovirus (HRV) infection is the cause of about one half of asthma and chronic obstructive pulmonary disease exacerbations. With more than 100 serotypes in the HRV reference set, an effort was undertaken to sequence their complete genomes so as to understand the diversity, structural variation, and evolution of the virus. Analysis revealed conserved motifs, hypervariable regions, a potential fourth HRV species, within-serotype variation in field isolates, a nonscanning internal ribosome entry site, and evidence for HRV recombination. Techniques have now been developed using next-generation sequencing to generate complete genomes from patient isolates with high throughput, deep coverage, and low costs. Thus relationships can now be sought between obstructive lung phenotypes and variation in HRV genomes in infected patients and potential novel therapeutic strategies developed based on HRV sequence. (J Allergy Clin Immunol 2010;125:1190-9.)

Key words: Asthma, chronic obstructive pulmonary disease, inflammation, virus

The human rhinovirus (HRV) is the primary causative agent of the common cold, and HRV infection appears to trigger approximately 50% of asthma and chronic obstructive pulmonary disease (COPD) exacerbations. Early HRV infection also appears to be a major risk factor for asthma in later life. These lower airway effects represent significant morbidity and mortality associated with HRV and were the impetus for us to sequence the full genomes of the archived reference set of 99 prototypic HRV serotypes. This provided an opportunity to ascertain the diversity of HRVs at the whole-genome level, to define commonalities and differences, to begin to make refined structure/function predictions related to pathogenesis and novel therapies, and to develop tools for rapid full-genome sequencing and analysis from patient isolates for epidemiologic studies. In this review the salient findings from these endeavors are presented, with an emphasis on application to asthma and COPD exacerbations.
HRV TAXONOMY AND GENOMIC FEATURES

The taxonomic classification of HRV has been recently revised (http://www.ictvonline.org). Previously, HRVs were placed in the family Picornaviridae, genus Rhinovirus, and each serotype was considered a species (e.g., hrV-16 and hrV-28). The current taxonomy maintains HRV in the Picornaviridae family, but the genus is Enterovirus, with 3 species: HRV-A, HRV-B, and HRV-C. Within each species there are multiple HRVs (variably designated as “serotypes,” “types,” or “strains”). Of note, the original approximately 100 HRVs collected from patient samples in the 1960s and 1970s (the reference or prototype set), which have been propagated and maintained by the American Type Culture Collection (ATCC), were in fact subjected to serotyping. Subsequently, as gated and maintained by the American Type Culture Collection, approximately 100 HRVs collected from patient samples in the 1960s and 1970s (the reference or prototype set), which have been propagated and maintained by the American Type Culture Collection (ATCC), were in fact subjected to serotyping. Subsequently, as sequencing technology has improved, distinct HRVs are being discovered but are no longer serotyped. For the work discussed here, we used the collection of HRVs from the ATCC because these are covered but are no longer serotyped. For the work discussed here, we used the collection of HRVs from the ATCC because these are frequently used by many investigators in in vitro and in vivo experiments and thus provide a collection relating phenotypes to an HRV with a known sequence. This reference set includes 74 HRV-A and 25 HRV-B serotypes, which have been assigned names by convention starting from “hrV-1.” The HRV-C species has only recently been recognized and currently consists of at least 11 types whose complete genomes are known. To date, these HRV-Cs have not been successfully infected into host cells ex vivo, and thus there has been a limited amount of virus available for sequencing or biologic studies. However, there is a growing body of evidence suggesting that HRV-C infections might be associated with greater respiratory impairment or symptoms.

HRV is a positive-sense single-stranded RNA virus of approximately 7,200 bp. The genome consists of a single gene, the translated protein of which is cleaved by virally encoded proteases to yield 11 proteins (Fig 1). The 5’ untranslated region (UTR) is typically approximately 650 bases, the open reading frame is approximately 6,500 bases (approximately 2,100 encoded amino acids), and the 3’ UTR consists of approximately 50 bases. At the 5’ terminal U, all HRVs are covalently linked to a small viral protein (VPg), which serves as a primer for genome replication. The 5’ UTR contains a number of structural and sequence elements necessary for gene translation and other functions (see below). After cleavage of the single polypeptide product of the open reading frame, various proteins assemble to form the capsid or carry out other specific functions related to replication. The capsid is composed of the VP4, VP2, VP3, and VP1 proteins arranged together as a unit (termed the protomer). The protomers are organized into 12 pentamers, which are linked to each other along the 2-fold symmetry edges. Thus the entire capsid is composed of 60 copies each of the 4 capsid proteins. The full icosahedral structure, as determined by means of X-ray crystallography, reveals a canyon in VP1 encircling each central plateau around the 5-fold axes of symmetry (see illustrations in references). Certain amino acids within these canyons provide the virion with attachment sites to the cell-surface intercellular adhesion molecule 1 receptor, to which 88 of the HRV-As and HRV-Bs bind. The other 11 (among the HRV-Cs) bind to the VLDL receptor. The cellular receptor for the HRV-Cs is not known. Within each VP1 protein, a hydrophobic pocket, which is among the known binding sites for any of several antiviral compounds, is accessible through the canyon.

The remaining portion of the genome open reading frame encodes proteases, a polymerase, VPg, and other proteins required for infection. Of note, the HRV 3D polymerase, which is necessary for synthesis of new genome, has no proofreading capacity, and therefore mutations at this step are assumed to be frequent. If so, then like poliovirus, a pool or “cloud” consisting of (1 or more) high-abundance HRV and multiple low-abundance mutated HRVs of various related sequences might be present during a given infection. For polio, this quasispeciation contributes to virulence. To date, the presence or relevance of nonclinical HRVs in a human infection is not known. Within the 3’ UTR...

GLOSSARY

**ARBITDOL:** An antiviral compound that has been used to treat influenza, rhinovirus, and hepatitis C.

**CAPSID:** The protein shell of the virus that protects the genome and allows entry into the host cells.

**ENVIROXIME:** An antiviral agent that inhibits viral replication by inhibiting the viral protein 3A.

**HIDDEN MARKOV MODELS:** A statistical model system that can be applied to bioinformatics for predicting and testing relationships. In this model the state is not visible, but the output dependent on the state is visible. Based on this, one can predict the likelihood that any given observation reflects the hidden state.

**INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1):** ICAM-1 is expressed on numerous cell types. Many rhinovirus types bind ICAM-1 expressed on nasal epithelial cells.

**NEIGHBOR-JOINING ALGORITHM:** A methodology used in bioinformatics that uses DNA or protein sequences to construct phylogenetic trees.

**OPEN READING FRAME:** The sequence of bases that could encode a protein. An open reading frame is located between the start codon (ATG and methionine) and the stop codon; any open reading frame can be read in 6 reading frames (in double-stranded DNA).

**PHYLLOGENETICS:** Phylogenetics uses sequencing data to evaluate the evolutionary relatedness between organisms.

**PLECONARIL:** An antiviral agent that integrates into the hydrophobic core of the picornavirus capsid.

**REVERSE TRANSATION:** Refers to the process of entering a protein sequence into a program that then turns the amino acid sequence back into a DNA nucleotide sequence.

**RUPINTRIVIR:** An antiviral agent that inhibits viral replication by inhibiting the rhinovirus 3C protease.

**UNIVERSAL PRIMERS:** Primers used in PCR and DNA sequencing that recognize sequence from all (or most) HRV strains.

**5’ UTR, 3’ UTR:** The UTR of mRNA located upstream and downstream of the coding sequence, respectively.

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are structural elements with features that appear to act in the regulation of transcription.22

**SEQUENCING THE REFERENCE SET OF HRVS**

Our initial approach for sequencing the ATCC reference set of HRVs was to devise universal primers that might be suitable for PCR amplification along the genomes for all the HRVs from the ATCC, as well as field samples. The original primers were designed from the 8 full-genome sequences that were available at that time and from limited sequence fragments of other HRVs. However, this approach failed to produce conditions that consistently provided amplicons for sequencing, and therefore instead we used a random, sequence-independent, single-primer shotgun sequencing method.6,23 Briefly, the new primers for reverse transcription (to generate cDNA) consisted of all possible combinations of the 4 nucleotides as 7-mers, each of which had a unique 5′ tag. From a reverse transcriptase reaction with all approximately 4,000 primers, there were matches to many sequences throughout each approximately 7,200-bp genome, and a large number of products were generated. PCRs with the tag sequence for priming provided amplicons that could be cloned into a sequencing vector. After transformation, approximately 300 colonies were picked for expansion, providing inserts of various sizes covering each genome, with substantial overlap in redundant sequences (Fig 2). The sequence of any areas in the genome in which there were gaps or coverage was minimal was filled in after picking additional clones or by specific PCRs primed by adjacent sequence. This approach yielded approximately 6-fold redundancy across each of the hrv genomes.24,25 Ultimately, we completed the full sequences of 88 HRV-As and HRV-Bs, including 10 HRVs from recent patient samples (here designated with an “fx” along with

**FIG 1.** Organization of the HRV genome. The genome consists of 5′ and 3′ UTRs and a single open reading frame encoding 1 protein, which is subsequently cleaved to form 11 viral proteins that act to form the capsid (VP4-VP1) or are required for viral replication. Adapted from Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, et al.6

**FIG 2.** Representative HRV sequence coverage by using the sequence-independent random primer method. Each horizontal line represents a contiguous sequence, ranging in size from approximately 50 to approximately 1000 bases. The vertical component represents the extent of redundancy for any given nucleotide or genome segment. The horizontal component represents the extent of the coverage of the genome.
the serotype). When our data were combined with those of 2 other groups who were also sequencing HRV, we had the first description of the full-genome set of the ATCC reference library, as well as the sequences of our additional field strains.

**STRUCTURE-BASED ALIGNMENT OF RNA AND AMINO ACID SEQUENCES**

An accurate alignment of the RNA sequences and the translated amino acid sequences is necessary to begin to define the commonalities and differences between the serotypes. The alignment is also critical for ascertaining phylogenetic relationships. We thus chose to use a molecular structure–based approach for the alignments so as to maximize potential structure/function relationships and phylogenetic inferences. For the proteins, the initial fits were by means of superimposition of the amino acids with known HRV and related virus structure maps derived from X-ray crystallography. In a stepwise manner, profiled hidden Markov models (HMMs) supplemented the founder set with remaining sequences. With these alignments, the open reading frame was established for each HRV by means of reverse translation, and the insertion/deletion regions were thereby identified. For the 5′ and 3′ UTRs, we used thermodynamically derived folding motifs as a template by which to progressively establish alignment. Here minimal energy hydrogen bonding configurations were calculated for each HRV. Superimposition of these 5′ and 3′ motifs provided the core consensus profiles for RNA alignments. Complete genomes were then reconstructed by concatenating the aligned 5′, coding, and 3′ RNA sequences. These alignments, which also include the previously sequenced HRV-A and HRV-B and the recently sequenced HRV-C, can be found in the supplementary data of Palmenberg et al.

**5′ UTR SEQUENCE ANALYSIS**

A number of intriguing findings came from comparative analysis of the 5′ UTR sequences from the 99 HRV-A and HRV-B and 7 HRV-C complete genomes. As was expected, we found the 5′ terminal portion of the 5′ UTR modeled into a cloverleaf for each HRV (Fig 3, A). The configuration of the cloverleaf appeared to be quite similar between species. In contrast, a pyrimidine-rich spacer tract just 3′ to the cloverleaf had a different nucleotide sequence for each HRV serotype from the reference set, and even within-serotype differences were observed when the reference samples were compared with recently obtained field isolates (Fig 3, B). The analogous position in the poliovirus genome (denoted 10b) is critical for neurovirulence, whereas aphthoviruses and cardioviruses have poly(C) or poly(UC) tracts in this position that are also required for virulence. If this same region in HRV has a similar function, the marked sequence variation we found might indicate that serotypes have significant differences in virulence and that virulence within a serotype might be actively changing. Adjacent to the spacer tract (moving 3′) is the internal ribosome entry site (IRES), which initiates translation for the open reading frame (moving 3′) is the internal ribosome entry site (IRES), which initiates translation for the open reading frame. Translation in enteroviruses through IRESs occurs by binding of the 40S ribosomal subunit to a 5′ triplet (e.g., AUG), with subsequent scanning through the intervening sequence to the initiator AUG. For all HRVs, we found that the minimal energy model revealed that the initiator AUG was invariably paired to a 5′ upstream AUG.
This pairing suggests a nonscanning IRES; that is, HRVs can use the proximity of the AUGs to orient the 40S ribosome to proceed with translation in a bait-and-switch fashion without the need for scanning. This mechanism might enhance HRV translation competitiveness. A similar pairing is not found with any other enterovirus.

**CODING SEQUENCE COMPARISONS**

The amino acid identities between any 2 hrvs is shown in matrix form in Fig 5. Readily apparent are the high degrees of identity between members of a given species (eg, orange and pink regions) and the lower identities when comparing across species (gray areas). It is also clear that there is heterogeneity within species, as represented by “islands” of discontinuity in the color scheme. This suggested that certain HRVs might cluster together in terms of relatedness and perhaps function. Phylogenetic trees were constructed by using the full genomes to assess relationships among HRVs. One such tree, constructed with a neighbor-joining algorithm, is shown in Fig 6. This approach might not be ideal for accurately defining the evolutionary path by which a given HRV arose (see below) but does provide an accurate model of the relationships between the various types. A poliovirus and 2 coxsackievirus genomes were used as the outgroups to root the tree. As shown in Fig 6, HRV-A, HRV-B, and HRV-C each form distinct major clades. It also appears that HRV-A and HRV-C share a common ancestor that is a sister group to the ancestral HRV-B. The early divergence of HRV-C and the presence of many serotypes within HRV-A and HRV-B species suggests evolutionary “space” within HRV-C, indicating that there might be many additional HRV-Cs yet to be discovered. We also note the distinct clustering and apparent early divergence of the small HRV-A clade denoted.
here as “clade D.” This might be indicative that these represent a fourth or newly emerging species (HRV-D). Within the larger HRV-A, there are clear miniclades that might represent groupings of HRVs with similar characteristics. The group starting with hrv-20 at the bottom of the figure and moving counterclockwise to hrv-80 represents one such miniclade. Similarly, clusters of serotypes are noted elsewhere, such as hrv-89 through hrv-88 and hrv-81 through hrv-01. Furthermore, within the large group extending from hrv-09 through hrv-100, there are several additional clusters of more than 5 members with apparent early divergence from an ancestral HRV. Similar clustering is seen with HRV-B (Fig 6) and will likely be noted for HRV-C as more types are sequenced. Some of this clustering can be seen from the amino acid comparison matrix, such as the orange section encompassing hrv-12 through hrv-89 of Fig 5, which represents the first 2 miniclades in Fig 6 discussed above. We compared the full-genome tree with other trees constructed using only the VP1 sequence, VP4+2 sequence, 3Dpol sequence, or IRES, with the null hypothesis being that these trees would not differ from the full-genome tree. However, all 4 of these alternative trees differed statistically from the full-genome tree (P values from approximately $10^{-6}$ to $10^{-100}$). This suggested that HRVs might have evolved with mutations throughout the genome rather than strictly at the most immunologically exposed regions.

**RECOMBINATION BETWEEN HRVS**

The nature of the diversity of HRVs was further explored by examining the potential for recombination. Unexpectedly, we found highly statistically significant evidence for recombination, as shown in Fig 7. In this example hrv-53 and hrv-80 each contributed portions of their genomes to hrv-46. As can be seen, there is a high degree of identity between hrv-80 and hrv-46 in the 5′ portions of the genomes up to approximately 3,600 b, which represents most of the 5′ UTR, VP4, VP2, VP3, and VP1. The remaining approximately 3,900 b of hrv-46 came from hrv-53, as indicated by the increased identity. Furthermore, the identities between the 2 parents (yellow line) is low, indicating that the recombination occurred between 2 relatively dissimilar hrvs (see location in the tree of Fig 5). Altogether, 23 HRVs in our total dataset of full-length genomes were found to originate through recombination events. Given that coinfection with 2 HRVs obviously occurs, these results provide compelling evidence for such conditions resulting in the creation of distinct new HRVs. What is currently not known is the frequency of such events, when these events might have occurred, and whether additional newer recombinants will be found as more hrvs from patient samples are sequenced. Interestingly, within our virus cohort, the degree of identity between the donated parent segments and the analogous daughter segments never approached 100%. In the
example shown, it peaked at approximately 65% and approximately 72% for each parental segment versus the daughter segment. This might suggest that these recombination events were distant as opposed to recent, such that subsequent to the recombination, many additional mutations were fixed in the respective genomes. However, we do not really know the within- or between-host mutation rate of HRV, and therefore even the terms recent and distant are at best descriptive. An alternative hypothesis that cannot be excluded based on the data at hand is that there might be additional HRVs in circulation that we do not know about that might have recently recombined. Under this scenario, the lack of higher identities simply reflects an incomplete dataset of circulating HRVs. Sequencing genomes from large numbers of current patient samples will help to address these types of issues and will further capture the extent of the diversity in the circulating pool of HRVs.

With the discovery of recombination, the use of phylogenetic methods that infer a strictly bifurcating tree becomes problematic if the goal of the analysis is to understand how a given hrv came to lie within a clade. There are techniques that allow for parallel or conflicting tracts of evolution reflecting recombination events or selection, essentially inferring relationships that represent more complex models of evolution.\textsuperscript{30,31} The output from such algorithms appears as a network rather than a tree. We recently performed such an analysis with our full-genome database\textsuperscript{32} and the clusters found with the neighbor-joining method (Fig 6) are entirely consistent with the network model.

FIELD ISOLATES

Although we had full-genome sequences from only 10 field samples recently obtained from patients (nasal secretions), it was useful to see the variations between these genomes and the analogous HRVs represented by the ATCC samples, given that they differed by more than 30 years in collection times. From our phylogenetic analysis (Fig 6), it was readily apparent that each field isolate was identifiably close to one of the reference serotypes (see hrv-89-f09, hrv-89-f08, and hrv-89 at the bottom of
the tree). Therefore these fit readily into the scaffold as minor variants in all cases. Nevertheless, up to approximately 800 base changes in RNA sequence were sometimes found between some of these recent isolates and their reference counterparts, representing approximately 10% of the genome. In terms of non-synonymous variations (those that change the amino acid), approximately 50 such variations were noted between some recent isolates and their analogous ATCC sample. We found no readily apparent pattern regarding regions of the HRV genome that were hypervariable or conserved within a serotype, but the sample size is inadequate for such an analysis. We sequenced 2 field isolates each of hrv-09, hrv-81, and hrv-89. These were all collected in 2005-2006, and interestingly, there were far fewer variations between the most recently collected isolates (within serotype) compared with their analogous older ATCC isolates. Although it is tempting to calculate a “mutation rate,” realistically, our sample size is simply too limiting to consider this analysis, and of course, one cannot exclude that the variant strains, albeit recently collected, were not also in circulation when the analysis, and of course, one cannot exclude that the variant strains, albeit recently collected, were not also in circulation when the ATCC samples were collected. It is important to note that one should not expect that all future patient isolates will necessarily be variants (however so defined) of one of the fully sequenced HRVs from the ATCC set. Indeed, within GenBank, one can find HRV-like sequences (typically far shorter than full-length sequences) that might represent novel types. As an example, we recently reported the full-genome sequence of an HRV (denoted hrv-A101) that fell within the HRV-A species but had only 76% nucleotide identity with its nearest neighbor (a lower value than virtually all other HRVs compared with their closest match) and was distinctly localized within the HRV phylogenetic network. Although specific definitions of an HRV type or strain have not yet been articulated by the International Committee on Taxonomy of Viruses (in progress), the totality of the evidence from this HRV genome is indicative that it is unique from those previously sequenced. GenBank searches showed partial sequences with high identity to hrv-A101 reported from isolates from China, Germany, Spain, Australia, Belgium, and the United States. Thus even within the “packed” HRV-A species, there are likely to be other types awaiting discovery. Given that recombination is prevalent, it will be important to have a full-genome sequence of new HRV isolates to place them into context within the group of known isolates.

**IMPLICATIONS FOR OBSTRUCTIVE LUNG DISEASE AND FUTURE DIRECTIONS**

The results discussed in this review provide opportunities to explore basic questions about HRV biology and the link between HRV infection and asthma or COPD pathophysiology. First, we now have a scaffold (the alignments and phylogeny) to integrate additional strains as they are identified into the 3 (or 4) well-defined HRV species. Furthermore, the pipeline methods have been developed to rapidly sequence the complete genomes of HRVs obtained from patient samples. Therefore the diversity of HRV at the full-genome level can now be ascertained within a community during a single cold season. Similarly, the mutation rate of an HRV, from onset to recovery, within a single subject could be determined, as well as how the same virus, when naturally transmitted to another person, might mutate in response to different host factors. The effects of concomitant bacterial or other viral infection or the upper airway microbiome could also be addressed.

With proper study design, it should be possible to begin to relate a clinical phenotype to an HRV genomic sequence. Among possible approaches, for example, consider a hypothetical study that might reveal that of 30 HRVs identified from a group of asthmatic subjects with symptomatic upper respiratory tract infection, only those who had an HRV belonging to a certain miniclade had an asthmatic exacerbation. Such a finding would lead to a scrutiny of those HRV genomes to ascertain which features promote the “proexacerbation” phenotype. Alternatively, it might be found that the most robust association is with a particular genome segment, which does not necessarily track with whole-genome phylogeny. Again, such a finding would prompt study of that region of the genome and the unique properties of the group that was associated with exacerbation. On an even finer scale, it might be found that certain variations (“polymorphisms”) within one or more parts of HRV genomes are the real determinants of exacerbation. Possibly, these HRVs could be found scattered throughout the HRV tree, even when a tree is constructed with small genome segments.

Some of the future studies outlined above, such as those examining mutation rates, obviously require the availability of full-genome sequences. In most of the other potential studies, our bias is to “start big.” That is, use all of the genetic information from a complete genome in these association studies with asthma

![Image](115x582 to 473x717)
phenotypes. This bias is based on our assumption that the HRVs evolve within the context of the full genome rather than as isolated segments without dependence on other parts of the genome. As more data are gathered, we might well be able to use shorter HRV sequences to identify those asthmatic subjects who will have exacerbations, but given the advances in high-throughput sequencing technology, there is little reason (now) to compromise a study by using limited HRV sequence information. Regardless, many samples are going to be required to carry out these analyses, and an effort by the International Rhinovirus Consortium (http://www.international-rhinovirus-consortium.org) to collect and sequence HRVs from adequately phenotyped subjects is underway.

The full set of genome sequence data, RNA structural predictions, phylogeny, and recombination results have multiple implications for development of anti-HRV therapeutics. Many attempts have been made to devise broad-spectrum efficacious drugs that act at 1 or more events in the HRV infection and replication cycles. Thus far, these efforts have taken a “one drug fits all” approach. Drugs targeting viral attachment (soluble intracellular adhesion molecule 1, pleconaril), viral fusion to the cell membrane (arbidol), capsid uncoating (pleconaril), capsid stabilizers (Ro-09-0410), viral replication (enviroxime), translation (IRES-based oligomers), and posttranslational processing (inhibition of proteases by agents such as rupintrivir) have been developed and studied in vitro and in clinical trials. Typically, these experiments show excellent antiviral effects in cell-culture systems but minimally significant or nonsignificant effects in human clinical trials. Even when a realized prophylactic effect to experimental HRV infection can be observed (such as a decrease of approximately 1-2 days of clinical symptoms, nasal viral titers, or secretions), further studies assessing prophylaxis or acute treatment of naturally occurring colds from HRV often show no significant effect.

The basis of these prior failures is not altogether clear. In some cases the route of administration (or the formulation) might have been suboptimal. However, many of these failures might be attributable to our overall lack of understanding of the basic biology of HRV infection in the human airway and the genetic diversity and real-time evolution of natural HRV strains.

The clinical experiences with pleconaril exemplify such issues.33,34 This agent showed inhibition of approximately 90% of HRV serotypes in vitro and in 2 placebo-controlled, double-blind clinical studies showed some degree of efficacy for treating naturally occurring colds. Observations included a decrease to illness resolution (7.9 vs 6.8 days for placebo), illness severity scores, and nasal mucus viral RNA at day 3 (97.7% decrease vs 90.3% decrease for placebo). Viral cultures and in vitro sensitivities of the isolated HRVs were also carried out at baseline and at day 3. Fewer pleconaril-treated patients had positive cultures at day 3 compared with placebo-treated patients (53% vs 72%, respectively). The clinical benefit appeared to correlate somewhat with the in vitro sensitivities at day 1: median effective concentration values of 0.38 μg/mL or less had reductions in symptom duration, whereas those with higher median effective concentration values showed no benefit. This latter group represented 60% of the subjects. Therefore although there was an overall benefit generically, clearly the drug was ineffective for a substantial subset of subjects. Moreover, in 10.7% of the pleconaril-treated subjects, the day 3 in vitro susceptibility was actually lower, and in 2.7% the virus was fully resistant to pleconaril. These latter data suggested the development of pleconaril-induced resistance during treatment in up to approximately 13% of patients. It would be highly informative to know the full-genome sequences (not just the serotype) of the HRVs that showed low sensitivity to pleconaril at day 1 and of those in which drug resistance developed. Potentially, pleconaril might be highly efficacious for a subset of HRVs based on a particular genomic sequence. A rapid diagnostic test could identify patients most likely to benefit based on HRV sequence at the onset of symptoms. In essence, this is a form of personalized medicine, in which the genome of the pathogen could be used to help guide treatment.

The potential for a successful vaccine seems remote at this time. Unfortunately, the very nature of HRV’s genetic diversity, fueled by dual-infection recombination and a high genome-specific mutation rate, are exactly the same parameters that work against effective vaccine development. The constant cocirculation of the 99 (100+) defined serotypes of HRV-A and HRV-B, let alone the added immunogenetic assortment of the numerous HRV-Cs, make it difficult to envision how a monovalent or even polyvalent formulation could cover this diversity. The sequences tell us there are no common epitopes conserved on the various capsid surfaces. The receptor-binding canyons are thought to be too deep and narrow for antibody recognition, and even if these regions could be targeted artificially, protective immunity against HRV is likely to require an IgA (mucosal) response and not the more common IgG (circulating) response, as elicited by other efficacious (eg, polio) picornavirus vaccines.

CONCLUDING REMARKS

In summary, the complete reference set of HRVs has now been sequenced at the full-genome level. The results provide new insights into HRV’s evolution and diversity and suggest levels of relatedness that might provide for signatures for asthma or COPD exacerbations. Structural predictions from the genomes might provide for novel therapeutics targeted to subsets of HRVs based on specific sequences.

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REFERENCES


