

## Absence of the Kilifi Mutation in the Rhinovirus-Binding Domain of *ICAM-1* in a Caucasian Population

LEEN VIJGEN, MARK VAN ESSCHE, and MARC VAN RANST

### ABSTRACT

Human rhinoviruses (HRV), responsible for approximately 60% of the common colds, are divided into two groups, according to their receptor specificity. The major group of HRVs gains access to human cells by binding to the intercellular adhesion molecule-1 (ICAM-1), whereas HRVs of the minor group use members of the low-density lipoprotein receptor (LDLR) family for cell entry. Previous studies confirmed that the HRV-binding region of *ICAM-1* is located in the amino-terminal immunoglobulin-like (Ig) domain 1, which is encoded by exon 2 of the *ICAM-1* gene. An A → T transversion in codon 29 of *ICAM-1* exon 2 causes a lysine to methionine substitution (K29M), and was found at a high frequency (33.2%) in Kilifi (Kenya), as well as in other African populations. In this study we examined whether polymorphisms in exon 2 of *ICAM-1* could be detected in a Caucasian population, assuming that these could be of importance in HRV binding. DNA from 100 healthy, unrelated, Belgian volunteers was obtained through a noninvasive swish-and-spit method. Using a primer set in the adjacent intron sequences, the full-length *ICAM-1* exon 2 was amplified by polymerase chain reaction (PCR), followed by direct sequencing of the PCR product. No polymorphisms could be demonstrated in exon 2 of the *ICAM-1* gene among all 100 tested individuals. The rhinovirus-binding Ig domain 1 of *ICAM-1* seems to be a highly conserved region in the Caucasian population.

### INTRODUCTION

THE COMMON COLD is one of the most widespread infectious diseases in humans, and approximately 60% of the cases are caused by rhinoviruses. Other pathogens associated with the common cold include coronaviruses, respiratory syncytial virus, and adenoviruses (Turner, 1997). Although this illness is characterized by relatively mild symptoms such as rhinorrhea, nasal congestion, and sneezing, the common cold has a high economic impact due to an enormous loss in productivity and high medical costs.

Human rhinoviruses (HRV) belong to the Picornaviridae, a family of small, nonenveloped, icosahedral viruses made of a protein capsid that encloses a single-stranded, positive-sense RNA molecule. The viral capsid contains 60 protomers, each of which is made up of four polypeptides, the viral proteins VP1–VP4. At the center of five protomers, a narrow surface depression, a “canyon,” with a highly conserved amino acid sequence has been demonstrated to be the interaction site between HRVs and their cellular receptors (Rossmann *et al.*, 1985; Ol-

son *et al.*, 1993). Until now, 102 different HRV serotypes have been identified, each characterized by its own specific antigens. According to their receptor specificity, HRVs can be divided into two groups (Abraham and Colonno, 1984). The 91 serotypes of the major group gain access to human cells by binding to the intercellular adhesion molecule-1 (ICAM-1) (Greve *et al.*, 1989; Staunton *et al.*, 1989), while the minor group, consisting of 10 serotypes, uses members of the low-density lipoprotein receptor (LDLR) family for cell entry (Hofer *et al.*, 1994). The receptor for one serotype, HRV 87, has yet to be characterized, but it is demonstrated that the presence of sialic acid on cellular receptors is essential for HRV 87 cell attachment and infection (Uncapher *et al.*, 1991).

ICAM-1 (CD54), the major group receptor, is a transmembrane glycoprotein of 505 amino acids, with a molecular mass of 80–114 kDa, depending on the degree of glycosylation, and is expressed on a wide variety of cell types. During inflammation, ICAM-1 plays a significant role in promoting cellular interactions by binding to its natural ligands, the  $\beta_2$  integrins: lymphocyte function-associated antigen (LFA-1; CD11a/

CD18) and macrophage-1 antigen (Mac-1; CD11b/CD18). The human *ICAM-1* gene is located on chromosome 19p13.3–p13.2 and consists of seven exons (Voraberger *et al.*, 1991). The *ICAM-1* glycoprotein belongs to the immunoglobulin gene superfamily, having five amino-terminal extracellular immunoglobulin-like (Ig) domains, with each domain encoded by a separate exon (Voraberger *et al.*, 1991). The binding site for human rhinoviruses is located within the first 88 amino acids of the amino-terminal Ig domain I of *ICAM-1*, which is encoded by exon 2 (McClelland *et al.*, 1991). In this exon, an A → T transversion in codon 29, causing a lysine to methionine substitution (K29M), was found at a high frequency (33.2%) in Kilifi (Kenya), as well as in other African populations (Fernandez-Reyes *et al.*, 1997; Craig *et al.*, 2000; Kun *et al.*, 1999).

In this study we examined whether polymorphisms could be detected in *ICAM-1* exon 2 in a Caucasian population of 100 unrelated, healthy individuals, assuming that these mutations could be of importance in HRV attachment to human cells. It is possible that interindividual differences in susceptibility to rhinovirus infections could correlate with genetic differences in the cellular rhinovirus receptors.

## MATERIALS AND METHODS

### Subjects

The study involved a Caucasian population of 100 healthy, unrelated individuals from the region of Flanders, the northern part of Belgium. Informed consent was obtained from all participants, and the study had the approval, based upon the guidelines from the World Medical Association's Declaration of Helsinki, of the Ethics Board of the University of Leuven (World Medical Association, 1996).

### DNA extraction

DNA samples were collected through a noninvasive "swish-and-spit" technique. Genomic DNA was acquired from oral epithelial cells, by rinsing the oral cavity with a 0.9% saline solution, after which DNA was extracted using an alkaline lysis procedure (Lench *et al.*, 1988).

### PCR amplification of *ICAM-1* exon 2

A 326-bp fragment, encompassing the full-length *ICAM-1* exon 2, was amplified by polymerase chain reaction (PCR), using a primer set in the adjacent intron sequences. The forward primer used was 5'-GTCGCCTCTCCCTCGTTTC-3' and 5'-TCTGCCTAGTCCAGCCCTC-3' was used as reverse primer (GenBank acc. no. AC011511). PCR amplification was performed in a volume of a 50- $\mu$ l reaction mix, with a concentration of 0.2  $\mu$ M of forward and reverse primer, 0.2 mM of nucleotides, 2.5 mM of MgCl<sub>2</sub>, and 1 unit of *Taq* polymerase (Applied Biosystems/Roche Molecular Systems, Belgium) at pH 9. PCR conditions were composed as follows: an initial denaturation at 95°C for 5 min, then 40 cycles of 30 sec at 95°C, 30 sec at 57°C, and 35 sec at 72°C, and a final elongation at 72°C for 7 min. The amplification reaction was performed in a Geneamp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). The resulting PCR products were vi-

sualized after electrophoresis, on an ethidium bromide-stained polyacrylamide gel.

### Sequence analysis

After purification of the PCR products with the QIAquick PCR purification kit (Qiagen, Westburg, The Netherlands), the purified products were cycle sequenced in forward and reverse direction using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Electrophoretic separation and detection were performed on an ABI PRISM 3100 Genetic Analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA).

## RESULTS AND DISCUSSION

The full-length *ICAM-1* exon 2 was PCR-amplified in 100 samples from unrelated Belgian individuals, in search for polymorphisms in the rhinovirus-binding Ig domain I of *ICAM-1*. Using primers located in the adjacent introns, this PCR reaction generated an amplicon of 326 bp, containing the complete 264-bp exon 2, which was subsequently sequenced. The resulting chromatograms were analyzed using the SeqMan multiple sequence alignment tool (LaserGene, DNASTar, Madison, WI). Consensus sequences were compared with a reference *ICAM-1* exon 2 sequence in GenBank (accession number X59287) using BLAST (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990). All the analyzed sequences were 100% identical to the reference sequence in GenBank.

Residues critical for HRV attachment are situated in the BC, CD, DE, and FG loops of the crystal structure of *ICAM-1* Ig domain I (Bella *et al.*, 1998; Casasnovas *et al.*, 1998; Kolatkar *et al.*, 1999). Previous studies showed that induced mutational changes in the BC, CD, DE, and FG loops of *ICAM-1* domain I could affect the ability to bind human rhinoviruses (Staunton *et al.*, 1990; Register *et al.*, 1991). Therefore, it is plausible that the presence of natural mutations in this HRV-binding region of *ICAM-1* could possibly influence HRV attachment and infection of human cells. The previously reported K29M mutation, also referred to as '*ICAM-1* Kilifi', lies at the end of the HRV-binding BC loop of *ICAM-1* domain I, making this polymorphism a possible candidate for altering the capacity of the major group HRVs to enter human cells (Fernandez-Reyes *et al.*, 1997; Bella *et al.*, 1998; Casasnovas *et al.*, 1998; Craig *et al.*, 2000). Because this mutation is very frequent in the African part of the world, it would be interesting to investigate its consequence on HRV infections in an African population.

The other Ig domains of *ICAM-1* might play an indirect role in rhinoviral entry, because they can affect the accessibility of the HRV interaction site, and polymorphisms in these parts might have some influence on viral attachment (Staunton *et al.*, 1990). Nevertheless, domain I of *ICAM-1* remains the most important site of rhinoviral contact. Several studies demonstrated that only domains 1 and 2 are sufficient for rhinovirus infection, and that the specificity for rhinovirus binding is entirely contained within the first 88 amino acids of domain I (McClelland *et al.*, 1991; Lineberger *et al.*, 1992; Staunton *et al.*, 1992).

No polymorphisms could be revealed in exon 2 of the

*ICAM-1* gene among all 100 tested Belgian individuals. Therefore, the rhinoviral binding domain 1 of *ICAM-1* seems to be a highly conserved region in a Caucasian population. The mechanisms of resistance to rhinovirus-induced common cold in some individuals remains unknown, although our results indicate that in a Caucasian population variability in the interaction site of the major group HRV receptor might not be a contributing factor. However, it is demonstrated that polymorphisms in this area of *ICAM-1* do occur in other populations (Fernandez-Reyes *et al.*, 1997; Kun *et al.*, 1999; Craig *et al.*, 2000). Further research in other populations might yield interesting results.

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Address reprint requests to:

Prof. Dr. Marc Van Ranst  
 Laboratory of Clinical and Epidemiological Virology  
 Department of Microbiology & Immunology  
 Rega Institute for Medical Research  
 Minderbroedersstraat 10  
 BE-3000 Leuven, Belgium

E-mail: marc.vanranst@uz.kuleuven.ac.be

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