UNIVERSITI PUTRA MALAYSIA

POTENTIAL USEFULNESS OF VIRAL CAPSID SURFACE PROTEINS (VP1, VP2, VP3 & VP4) FOR VACCINATION AGAINST COMMON COLD

ALSHRARI, AHMED SUBEH D

FPSK(p) 2015 16

POTENTIAL USEFULNESS OF VIRAL CAPSID SURFACE PROTEINS (VP1, VP2, VP3 & VP4) FOR VACCINATION AGAINST COMMON COLD

By

ALSHRARI, AHMED SUBEH D

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

August 2015

COPYRIGHT

بسم الله الرحمن الرحيم

This thesis is dedicated to

my late father, Subeh Daish Al-Hamlan.

And

my mother, Thaklah Maish Al-Hamlan,

It is also dedicated to

my children, Faisal, Faris, Abdullah and Norah, for their care, love, understanding, and patience.

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirements of the degree of Doctor of Philosophy

POTENTIAL USEFULNESS OF VIRAL CAPSID SURFACE PROTEINS (VP1, VP2, VP3 & VP4) FOR VACCINATION AGAINST COMMON COLD

By

ALSHRARI, AHMED SUBEH D

August 2015

Chairman: Prof. Zamberi Sekawi, MD, MPath
Faculty: Medicine and Health Sciences

Rhinoviruses (RVs) represent the most important etiological agents of the common cold and it is responsible for about two-thirds of acute exacerbations of chronic bronchitis, asthma and chronic obstructive pulmonary disease (COPD) in both children and adults. At present, there is no effective and approved antiviral therapies for either the prevention or treatment of diseases caused by RV infections. Furthermore, there are more than 100 types of RVs with high sequence variability hindering the progression of vaccine development. Bioinformatics tools, combined with the availability of complete genome sequence of all known RV types, provides a unique opportunity to enhance the optimal selection of potential immune targets. *In vitro* production or synthetic versions of these targets could be a possible alternative approach to the vaccine of choice. This study was carried out with the aim to develop a pan-serotypic vaccine that is capable of inducing the production of cross-reactive antibodies that cover all or most of the RV serotypes.

Firstly, a bioinformatics analysis was carried out to characterise the capsid proteins (VP1, VP2, VP3 and VP4) of all known RV serotypes and to predict potential immune motifs. In brief, complete protein sequences of each of the 100 distinct RV genomes were downloaded from the GenBank database. The sequences obtained were grouped based on their original classification [RV-A divided into two sub-groups, minor LDLR(n=10) and major ICAM(n=65), and RV-B group (n=25)]. Upon grouping, sequence editing was carried out using a number of software in order to study each protein individually. The edited protein sequences were then aligned and analysed for sequence conservation, variability and to generate consensus sequences and distance matrices. This led to determining the relations between strains and identifying the ideal ones that are highly identical to others. Conserved motifs consisting at least nine-mers common across all RV-A or B serotypes (minor/major receptor) and exhibiting at least 80% representation were selected and synthesized chemically. These peptides were used alone or in combination to vaccinate groups of rabbits. On the other hand, four tagged full-length genes coding the capsid proteins of an ideal strain (HRV-74), VP1, VP2, VP3 and VP4, whose codon uses were optimized, were constructed and cloned *in vitro*.

Upon expression, the purified recombinant proteins adsorbed into incomplete Freund's adjuvant (IFA) as a single or combined proteins were also administered subcutaneously to other groups of rabbits. The responses and cross-reactivity of the specific immunoglobulin M (IgM) and G (IgG) to the peptides, proteins and whole viruses were measured by in-house indirect enzyme-linked immunosorbent assay (ELISA). Moreover, *in vitro* cross-neutralizing antibody titres against several variant strains of RV were also measured.

Based on the bioinformatics analysis, 7, 8, 5 and 3 conserved regions were found among minor receptor serotypes for VP1, VP2, VP3 and VP4, respectively. The analysis of RV-A ICAM-receptor serotypes showed 3 conserved regions in each of VP1, VP2 and VP4, while 4 conserved regions were found upon alignment of VP3 sequences, respectively. The study also showed that the capsid protein of HRV-B contained at least one conserved site upon multiple sequences alignments of each protein separately. Furthermore, the analysis revealed that 72% of VP4 sequence (69 amino acids in length) as highly conserved among the RV-A major receptor group, but VP3 did not show well conserved regions. The current study also showed that VP4 sequences of the minor receptor groups (n=10) contained three highly conserved sites which accounted for 85% of its total length. RV-B VP4, in contrast, contained less conserved regions which exhibited only 25% of the protein's total length. Upon multiple sequence alignment of all RV-A, three highly conserved region were identified for each of the VP1, VP2 and VP4, while VP3 did not contain any.

Based on distance matrices analysis, HRV-74 was found to be the ideal strain for vaccine development. VP1 amino acid sequence of HRV-74 was found to be identical by 80% or more of 22 serotypes, with a median identity of 75% within the RV-A group. Also, the analysis revealed HRV-74 as having the highest homology (86%) to the VP1 consensus sequence of all RV-A. A further analysis showed that HRV-74 is fully identical (100%) to the consensus sequence of RV-A VP4. Therefore, HRV-74 has been considered as the source genetic information of the recombinant proteins produced in this study.

Antibodies raised to the synthetic peptides exhibited cross-reactivity against the corresponding recombinant proteins and antigenically distinct RV strains coated on plates via ELISA assay. Moreover, the specific immunoglobulin G (IgG) response to the peptides given in combination exhibited greater reactivity. Interestingly, the anti-peptide antibodies obtained exhibited a cross-neutralizing activity for different RV strains *in vitro*. In addition, the induced antibodies against recombinant proteins also reacted successfully with relevant proteins and with whole virus particle (HRV-74) and other variant strains, as shown by ELISA. They also showed strong cross-neutralizing ability against various variants of RVs.

Based on the antibody cross-reactivity and neutralization towards different studied serotypes, the selected RV strain HRV-74 seemed to be the type of choice for developing RV broad protective vaccine and multiple RVs antibody based on detection assay. The findings have indicated that the peptides corresponding to the conserved region of the RV capsid proteins are potent immunogenic and suggest that their combination is crucial for extending the cross-protection against variant RVs.

Such an alternative approach may raise hope for designing a novel broad-protective vaccine towards non-cultivable, hyper variable pathogen.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POTENSI KEPENGGUNAAN PROTEIN *VIRAL CAPSID SURFACE* (VP1, VP2, VP3 & VP4) SEBAGAI VAKSINASI MELAWAN DEMAM SELSEMA

Oleh

ALSHRARI, AHMED SUBEH D

Ogos 2015

Pengerusi: Prof. Zamberi Sekawi, MD, MPath
Fakulti: Perubatan dan Sains Kesihatan

Rhinoviruses (RVs) mewakili agen etiologi yang paling penting bagi demam selsema dan ia bertanggungjawab terhadap dua pertiga eksaserbasi akut bagi bronkitis kronik, asma dan penyakit penghalang pulmonary kronis (COPD) bagi kanak-kanak dan orang dewasa. Sehingga kini tiada terapi antivirus yang efektif atau yang diakui berupaya sama ada untuk mencegah atau merawat penyakit yang disebabkan oleh jangkitan virus RV. Tambahan pula terdapat lebih daripada 100 jenis virus RV yang mempunyai jujukan kebolehupayaan yang tinggi dalam menghalang perkembangan vaksin. Penggunaan alatan bioinformatik bersama jujukan genom yang lengkap yang sedia ada bagi kesemua jenis RV yang diketahui, menghasilkan peluang optimum yang unik dalam meluaskan seleksi bagi sasaran yang berpotensi imun. Penghasilan *in vitro* atau versi sintetik bagi sasaran-sasaran ini berupaya menjadi satu pendekatan alternatif terhadap vaksin yang dipilih. Objektif kajian ini dibuat adalah untuk menghasilkan satu vaksin *pan-serotypic* yang berupaya untuk menggalakkan penghasilan antibodi yang tindak balas silang meliputi kesemua atau sebahagian besar serotaip RV.

Kajian dimulakan dengan satu analisis bioinformatik untuk mengenalpasti protein kapsid (VP1, VP2, VP3 dan VP4) bagi kesemua serotip RV. Prosedur ini juga bertujuan untuk meramal motif yang berpotensi imun. Secara ringkas jujukan protein yang lengkap bagi setiap 100 genom RV yang berbeza telah dimuat turun dari pangkalan data *GenBank*. Jujukan yang diperolehi telah dikumpulkan berdasarkan klasifikasi asal mereka [RV-A dibahagi kepada dua kumpulan kecil, minor LDLR (n=10) dan major ICAM (n=65), dan RV-B kumpulan (n=25)]. Setelah diklasifikasikan, pengeditan jujukan telah dilakukan menggunakan perisian komputer untuk mengkaji setiap protein secara individual.

Jujukan protein yang telah diedit kemudian dijajarkan dan dianalisa bagi mengekalkan jujukan, variability dan untuk menghasilkan jujukan yang konsensus serta jarak matriks. Ini menentukan hubungan di antara regangan dan juga mengenalpasti regangan yang mempunyai persamaan yang paling identikal dengan yang lain. Motif-motif yang dikekalkan mempunyai sekurang-kurangnya *nine-mers* lazim merentasi kesemua serotaip RV-A atau B (reseptor minor/major) dan

menunjukkan sekurang-kurangnya 80% daripadanya telah dipilih dan disintesis secara kimia. Peptida ini telah digunakan secara individu atau kombinasi sebagai vaksin ke atas kumpulan-kumpulan arnab. Sementara itu, empat gen *tagged full-length* mengkodkan protein kapsid bagi regangan yang ideal (HRV-74), VP1, VP2, VP3 dan VP4, di mana penggunaan kodon telah dioptimumkan, disusun semula dan diklon secara *in vitro*. Setelah dianalisa, rekombinan protein yang diserap ke dalam *Freund's adjuvant (IFA)* yang tidak lengkap sebagai protein individu atau kombinasi telah digunakan secara subkutan terhadap kumpulan-kumpulan arnab yang lain. Tindakbalas dan tindak balas silang bagi spesifik immunoglobulin M (IgM) dan G (IgG) ke atas peptida protein dan keseluruhan virus telah di ukur oleh *enzyme-linked immunosorbent assay (ELISA)* dalaman secara tidak langsung. Selain itu, *in vitro* balas yang meneutralkan titres antibodi terhadap beberapa variasi RV juga telah diukur.

Berdasarkan analisis bioinformatik 7, 8, 5 dan 3 kawasan-kawasan terpelihara telah ditemui di antara serotaip reseptor minor bagi VP1, VP2, VP3 dan VP4, masing-masing. Analisis serotaip reseptor RV-A ICAM menunjukkan terdapat 3 kawasan yang terpelihara dalam setiap satu daripada VP1, VP2 dan VP4. Sementara itu 4 kawasan terpelihara telah ditemui dengan menjajarkan jujukan VP3 masing-masing. Kajian ini juga menunjukkan protein kapsid bagi HRV-B mengandungi sekurang-kurangnya satu kawasan terpelihara apabila penjajaran jujukan dilakukan beberapa kali bagi setiap protein secara berasingan. Tambahan pula analisis menunjukkan bahawa 72% daripada jujukan VP4 (yang mempunyai 69 asid amino panjang) sebagai sangat terpelihara di antara kumpulan reseptor major RV-A. Walau bagaimana pun VP3 tiada menunjukkan kawasan yang terpelihara. Kajian ini juga mendapati bahawa jujukan VP4 bagi kumpulan reseptor minor (n=10) mengandungi tiga kawasan terpelihara yang menyumbang 85% daripada jumlah panjangnya. Sebaliknya, RV-B VP4 mempunyai kawasan yang kurang terpelihara iaitu hanya 25% daripada jumlah panjang keseluruhan protein. Apabila penjajaran jujukan dilakukan beberapa kali terhadap kesemua RV-A, tiga kawasan yang sangat terpelihara telah dikenal pasti bagi setiap satu daripada VP1, VP2 dan VP4, manakala VP3 pula tidak mengandungi apa-apa.

Jarak analisis matriks telah mendapati HRV-74 sebagai regangan yang ideal bagi perkembangan vaksin. Jujukan asid amino VP1 bagi HRV-74 didapati identikal sebanyak 80% atau lebih daripada 22 serotaip yang mempunyai identiti median sebanyak 75% dalam kumpulan RV-A. Analisis juga mendapati HRV-74 mempunyai homology paling tinggi (86%) terhadap jujukan konsensus VP1 bagi kesemua RV-A VP4. Analisis selanjutnya menunjukkan bahawa HRV-74 identikal sepenuhnya (100%) terhadap jujukan konsensus bagi RV-A VP4. Oleh itu, HRV-74 telah diambil kira sebagai sumber informasi genetik bagi protein rekombinan yang dihasilkan dalam kajian ini.

Antibodi yang ditingkatkan kepada peptida sintetik menunjukkan tindak balas silang terhadap protein rekombinan dan regangan antigenetik RV yang berbeza yang berselaput di atas permukaan piring melalui ELISA assay. Tambahan pula, immunoglobulin G (IgG) yang spesifik bertindakbalas terhadap peptida yang diberi di mana kombinasi tersebut menunjukkan tindakbalas yang lebih besar. Menariknya, antibodi anti-peptida yang diperolehi menunjukkan aktiviti peneutralan balas bagi regangan RV *in vitro* yang berbeza. Disamping itu penambahan antibodi terhadap

protein rekombinan juga berjaya bertindakbalas ke atas protein yang relevan dan juga terhadap keseluruhan partikel virus (HRV-74) serta regangan varian yang lain seperti ditunjukkan oleh ELISA. Selain itu didapati juga kebolehupayaan peneutralan balas yang kuat terhadap pelbagai varian RV.

Berdasarkan antibodi tindak balas silang dan peneutralan terhadap kajian serotaip yang jauh berbeza, regangan RV yang dipilih iaitu HRV-74 seperti merupakan pilihan bagi perkembangan vaksin protektif RV yang luas serta pelbagai antibodi RV berdasarkan pengesanan assay. Dapatan kajian menunjukkan peptida yang bertindakbalas terhadap kawasan terpelihara oleh protein kapsid RV adalah poten imunogenik dan kombinasi ini penting bagi meluaskan kawalan balas terhadap varian RV. Pendekatan alternatif sebegini berkemungkinan dapat meningkatkan harapan dalam penghasilan vaksin baru yang mempunyai kawalan yang luas terhadap pathogen yang tidak boleh dibiak dan sangat mudah berubah.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to the creator deity of the universe, for giving me the strength and courage to complete this thesis.

I would also like to convey my deepest appreciation to my supervisor, Professor Dr. Zamberi Sekawi for his invaluable suggestions, encouragement and guidance. I appreciate Prof. Dr ZamberiSekawifor his support and patience during this research work.

I would also like to express my deepest thanks and admiration to my Co-supervisor, Prof. Dr. Abdul Rahman Omar and Associate Professor Dr. Chong Pei Pei, for their valuable advices, discussions and comments on this research. Also thanks for their guidance and ideas for bringing the thesis prepared.

I also want to take this opportunity to dedicate my appreciation to Dr. Alreshidi Mateq Ali, Dr. Hudu A Shuaibu, Dr. Nabil Harmal, Mr. Mohammad Reza, Mr. Ramli and Dr. Alsharari, Fahad M. for their helps, efforts and supports.

I would like also to express my special thanks to all my fellow friends and members of Clinical and Molecular Virology Laboratory and Medical Microbiology laboratory for their kind support throughout my study. I am extremely grateful for their ideas and efforts.

I certify that a Thesis Examination Committee has met on 12 August 2015 to conduct the final examination of Alshrari, Ahmed S on his thesis entitled "Potential Usefulness of Viral Capsid Surface Proteins (VP1, VP2, VP3 & VP4) for Vaccination Against Common Cold" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Vasantha Kumari Neela, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Rukman bin Awang Hamat, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Md. Mostafizur Rahman, PhD
Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

Patrick Cy Woo, PhD
Professor
University Pathology Building
Hong Kong
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 August 2015

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.
The members of the Supervisory Committee were as follows:

Zamberi Sekawi, **MD, MPath**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Abdul Rahman Omar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Chong Pei Pei, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- x this thesis is my original work
- x quotations, illustrations and citations have been duly referenced
- x the thesis has not been submitted previously or comcurrently for any other degree at any institutions
- x intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- x written permission must be owned from supervisor and deputy vice chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- x there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Alshrari, Ahmed Subeh D. (GS32158)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee: Zamberi Sekawi, MD, MPath	Name of Member of Supervisory Committee: Chong Pei Pei, PhD

Signature: _____
Name of Member of Supervisory Committee: Abdul Rahman Omar, PhD

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iv
ACKNOWLEDGEMENTS		vii
APPROVAL		viii
DECLARATION		x
LIST OF TABLES		xvi
LIST OF FIGURES		xvii
LIST OF ABBREVIATIONS		xxii
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	4
	2.1 Overview of Picornaviruses4	
	2.2 Rhinoviruses	6
	2.2.1 Classification	6
	2.2.2 Viral Properties	8
	2.2.2.1 Structure and Genomic Organization	8
	2.2.2.2 Viral Capsid	9
	2.2.3 Virus Receptors	10
	2.2.4 Virus Replication	10
	2.2.5 Transmission	11
	2.2.6 Epidemiology	11
	2.2.6.1 Geographical Distribution and Seasonal Pattern	11
	2.2.6.2 Age Distribution	12
	2.2.6.3 Clinical Presentation and Burden	13
	2.2.7 Immunity	14
	2.2.8 Antiviral Agents	16
	2.2.8.1 Inhibitor of Virus Attachment	16
	2.2.8.2 Viral Proteases	17
	2.2.9 Vaccination	18
	2.2.9.1 Challenges in Designing Vaccines against RV Infections	18
	2.2.9.2 Criteria of an Ideal Vaccine against Rhinovirus	20
3	**MATERIALS AND METHOD**	22
	3.1 Overview of the project methods and stages	22
	3.2 Bioinformatics approach	24
	3.2.1 Overview of the approach and data collection	24
	3.2.2 Grouping of sequences and identification of conserved region	27
	3.2.3 Entropy calculation	27
	3.2.4 Phylogenetic tree construction	27
	3.3 Preparation of constructs and production of RV recombinant capsid Proteins (VP1, VP2, VP3 and VP4)	28

3.3.1 Codon optimization and gene synthesis 28
3.3.2 Cloning and expression of the full-length codon-optimized VP1-4 recombinant proteins 29
3.3.3 Extraction of recombinant proteins 30
3.3.4 Protein purification 30
3.4 Protein assays 30
3.4.1 Proteins concentrations 31
3.4.2 Protein purity 31
3.4.3 Staining protein gels 31
3.4.4 Western Blotting 32
3.5 Synthetic peptides 35
3.6 Production of antibodies against recombinant VP1, VP2, VP3 and VP4 proteins and synthetic peptides 34
3.6.1 Ethics statement 34
3.6.2 Immunization of animals 34
3.7 Cell Culture and RV propagation 36
3.7.1 Growth and maintenance of H1-HeLa Cells 36
3.7.2 Virus propagation 37
3.7.3 Total RNA extraction 38
3.7.4 Measurement of RNA concentration and purity 39
3.7.5 Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) 39
3.7.6 PCR Amplification of the cDNA 39
3.7.7 Agarose gel electrophoresis of RT-PCR product 40
3.7.8 PCR and RT-PCR products purification 40
3.7.9 Sequencing Analysis40
3.8 Virus titration and determination of tissue culture infectious dose (TCID50) 41
3.9 Immunoassay experiments 41
3.9.1 Investigation of antigen-specific rabbit antibodies using indirect enzyme-linked immunosorbent Assay (ELISA) 41
3.9.2 Cross-reactivity of rabbits anti-synthetic-peptides with the recombinant proteins and vice versa by indirect ELISA 42
3.9.3 Reactivity and cross-reactivity of the rabbit anti-proteins and rabbit anti-peptides antisera with virus particles by indirect ELISA 42
3.10 Rhinovirus Neutralization Assay In vitro 43

4 RESULTS 44
4.1 Bioinformatics analysis 44
4.1.1 Sequence analysis of VP1 Amino Acids of all RV prototype strains 44
4.1.1.1 Sequence characterization of VP1 44
4.1.1.2 Conserved motifs of Rhinovirus VP1 protein 45
4.1.1.3 Phylogenetic analysis of rhinovirus prototypes strains based on VP1 amino acid sequences 48

4.1.1.4 Consensus sequences of VP1compared with HRV-74 VP1 51
4.1.1.5 Phylogenetic tree of VP1 amino acid sequences of RV-A 53
4.1.2 Sequence analysis of VP2 Amino Acids of all RV prototype strains 53
4.1.2.1 Sequence characterization of VP2 53
4.1.2.2 Conserved motifs of Rhinovirus VP2 protein 57
4.1.2.3 Consensus sequences compared with HRV-74 VP2 60
4.1.2.4 Phylogenetic tree of VP2 amino acid sequences of RV-A 61
4.1.3 Sequence analysis of VP3 amino acids of all RV prototype strains 63
4.1.3.1 Sequence characterization of VP3 63
4.1.3.2 Conserved Motifs of Rhinovirus VP3 Protein 65
4.1.3.3 Consensus sequences of VP3 compared with HRV-74 Protein 68
4.1.3.4 Phylogenetic tree of VP3 amino acid sequences of RV-A 70
4.1.4 Sequence analysis of VP4 amino acids of All RV prototype strains 71
4.1.4.1 Sequence characterization of VP4 71
4.1.4.2 Conserved motifs of Rhinovirus VP4 protein 72
4.1.4.3 Consensus sequences of VP4 compared with HRV-74 Protein 73
4.1.4.4 Phylogenetic tree of VP4 amino acid sequences of RV-A 76
4.1.5 Selection of serotypes for immunoreactivity studies 77
4.2 Gene design, synthesis and expression vector construction for production of HRV-74 capsid recombinant proteins (VP1,VP2,VP3 and VP4) 78
4.2.1 Codon optimization and genes synthesis 78
4.2.2 Analysis of cloning and transformation of the full-length codon-optimized VP11-4 recombinant genes 80
4.2.3 Analysis of the recombinant fusion proteins 82
4.2.4 Proteins purity and concentrations 87
4.3 Synthetic peptides 88
4.4 Propagation of RVs 90
4.4.1 Detection of RV by RT-PCR 90
4.5 Induction of Antigen-Specific immune response in animals 92
4.5.1 Immune responses to recombinant proteins 92
4.5.2 Immune responses to synthetic peptides 102
4.6 Cross-reactivity of induced antibodies with their corresponding antigens and with various variant RV strains 106
4.7 Neutralization and cross neutralizing assay of Rhinovirus in vitro 111

5 DISCUSSION 123

6 CONCLUSIONS 136

REFERENCES 138
APPENDICES 156
BIODATA OF STUDENT 192
LIST OF PUBLICATIONS 193

LIST OF TABLES

Table		Page
2.1	Taxonomic structure of the family *Picornaviridae*	5
3.1	Dataset of the Rhinovirus prototype strains collected from the public database records	25
3.2	Profile of full-length codon-optimized VP1-4 sequences encoding native HRV-74 capsid proteins	29
3.3	A list of the synthetic peptides used for the experiment	33
3.4	Batches of the experimental animals and the corresponding antigens	35
3.5	Immunization procedure, dose and blood collection times	36
3.6	Selected serotypes represent different branches of the RV phylogeny	38
3.7	Set of primers used to amplify the VP4/VP2 region	40
4.1	Conserved sequences identified within RV-VP1 capsid proteins	46
4.2	RV serotype pairs with VP1 **DPLRQDFLGLGHQWLWRI•**	49
4.3	RV-**SVHURWSHSDLUVZLWK9DPLRQDFLGLGHQWLWRI•**	56
4.4	RV-**VHURWSHSDLUVZLWK9DPLRQDFLGLGHQWLWRI•**	56
4.5	Conserved sequences identified within RV-VP2 capsid proteins	58
4.6	RV serotype pairs **ZLWK9DPLRQDFLGLGHQWLWRI•**	64
4.7	Conserved sequences identified within RV-VP3 capsid proteins	66
4.8	RV-A serotypes whose identical by 100% to HRV-74 based on VP4 aa	72
4.9	Conserved motifs identified within RV-VP4 capsid protein	73
4.10	Dataset of selected serotypes represent different branches of the RV phylogeny	77
4.11	Profile of full length codon-optimized VP1-4 sequences encoding native HRV-74 capsid proteins.	78
4.12	Codon distribution of VP1, VP2, VP3 and VP4 sequences	79
4.13	Profile of the recombinants proteins based on purity and concentration	87
4.14	Dataset of the synthetic peptides used for the experiment	89

LIST OF FIGURES

Figure		Page
2.1	Phylogenetic tree showing the relationships between all known RV serotypes.	7
2.2	Structure of typical RV genome.	8
2.3	The icosahedral structure of the rhinovirus capsid.	9
2.4	Viral replication in airway epithelial cells.	10
3.1	Methodology chart illustrates the key steps and stages involved in this project.	23
3.2	A general overview of the bioinformatics approach.	24
4.1	The length of VP1 protein (aa) for all RV prototype strains.	44
4.2	A major gaps inserted to facilitate VP1 alignment for RV-A and -B.	45
4.3	Entropy plot of the full-length VP1 amino acid sequence alignment of all RV-A serotypes (n=75).	47
4.4	Entropy plot of the full-length VP1 amino acid sequence alignment of all RV-B serotypes (n=25).	47
4.5	Median identity of VP1 aa of each serotype within RV-A.	50
4.6	Similarity of HRV-74 VP1 (aa) with other serotypes within RV-A.	50
4.7	VP1 amino acid identity in RV-A prototype strains (n=75) compare with the identity of VP1 of HRV-74 across the respective group.	51
4.8	Alignment of consensus of the RV-A VP1 sequences and HRV-74 VP1 sequence.	52
4.9	Phylogenetic tree of RV-A serotypes based on VP1 sequence.	54
4.10	Median identity of VP2 aa of each serotype within RV-A.	55
4.11	Pairs identity of HRV-74 VP2 (aa) with other serotypes within RV-A.	55
4.12	Entropy plot of the full-length VP2 amino acid sequence alignment of all RV-A serotypes.	59
4.13	Entropy plot of the full-length VP2 amino acid sequence alignment of all RV-B serotypes.	59

4.14	Alignment of consensus of the RV-A VP2 sequence and HRV-74 VP2 sequence.	60
4.15	Alignment of consensus of the RV-B VP2 sequence and HRV-74 VP2 sequence.	61
4.16	Phylogenetic tree of RV-A serotypes based on VP2 amino acid sequence.	62
4.17	Similarity of HRV-74 VP3 (aa) with other serotypes within RV-A.	65
4.18	Entropy plot of the full-length VP3 amino acid sequence alignment of all RV-A serotypes.	67
4.19	Entropy plot of the full-length VP3 amino acid sequence alignment of all RV-B.	67
4.20	Similarity of VP3 (aa) consensus sequence with all serotypes within RV-A.	68
4.21	Alignment of consensus of the RV-A VP3 sequence and HRV-74 VP3 sequence.	69
4.22	Alignment of consensus of the RV-B VP3 sequence and HRV-74 VP3 sequence.	69
4.23	Phylogenetic tree of RV-A serotypes based on VP3 amino acid sequence.	70
4.24	Median identity of VP4 aa of each serotype within RV-A.	71
4.25	Similarity of HRV-74 VP4 (aa) with other serotypes within RV-A.	72
4.26	Alignment of consensus of the RV-A VP4 and HRV-74 VP4 sequence.	73
4.27	Entropy plot of the full-length VP4 amino acid sequence alignment of all RV-A serotypes.	74
4.28	Entropy plot of the full-length VP4 amino acid sequence alignment of all RV-B serotypes.	75
4.29	Alignment of consensus of the RV-B VP4 and HRV-74 VP4 sequence.	75
4.30	Phylogenetic tree of RV-A serotypes based on VP4 sequence.	76
4.31	*E. coli* BL21 colonies after transformation of constructed cloning vectors.	80

4.32	Representative results show analysis of single colonies by PCR.	81
4.33	Representative result of purified constructed expression vectors.	81
4.34	Representative results show SDS-PAGE profile of *E .coli* BL21 proteins transformed with pET21a-VP1.	83
4.35	Representative results show SDS-PAGE profile of *E .coli* BL21 proteins transformed with pET21a-VP2.	84
4.36	Representative results show SDS-PAGE profile of *E .coli* BL21 proteins transformed with pET21a-VP3.	85
4.37	Representative results show SDS-PAGE profile of *E .coli* BL21 proteins transformed with pET21a-VP4.	86
4.38	Representative results for propagation of ATCC RVs using H1HeLa cell (magnification of 200x).	91
4.39	Representative results for amplification of VP4/VP2 fragments of RVs strains using RT-PCR.	92
4.40	Specificity of produced antibodies against recombinant VP1 and VP4 proteins.	93
4.41	Anti-VP1protein IgM antibodies reacted specifically with recombinant VP1 protein.	94
4.42	Anti-VP1protein IgG antibodies reacted specifically with recombinant VP1 protein.	94
4.43	Anti-VP4 protein IgM reacted specifically with recombinant VP4 protein.	95
4.44	Anti-VP4 protein IgG antibodies reacted specifically with recombinant VP4 protein.	95
4.45	Anti-VP2 IgM antibodies raised against recombinant VP2 or combination of VP1/VP2 proteins reacted specifically with recombinant VP2 protein.	96
4.46	Anti-VP1 IgM antibodies raised against combination of recombinant VP1/2 and VP1/3 reacted specifically with recombinant VP1 protein.	96
4.47	Anti-VP3 IgM antibodies raised against recombinant VP3 or combination of VP1/3 reacted specifically with recombinant VP3 protein.	97
4.48	Anti-VP1/2 and anti-VP1/3 IgM antibodies raised against combination of recombinant VP1/VP2 and VP1/VP3.	97

4.49	Anti-VP1/VP4 IgM antibodies raised against a combination of recombinant VP1 and VP4 proteins.	98
4.50	Anti-VP1/VP4 IgM antibodies raised against a combination of recombinant VP1 and VP4 proteins.	98
4.51	Anti-VP2 & anti-VP1/2 IgM antibodies raised against VP2 protein and combination of recombinant VP1/2 proteins.	99
4.52	Anti-VP3 & anti-VP1/3 IgM antibodies raised against VP3 protein and combination of recombinant VP1/3 proteins.	99
4.53	IgM antibodies raised against combination of (VP1-4) recombinant proteins.	100
4.54	IgG antibodies raised against combination of (VP1-4) recombinant proteins.	101
4.55	Specificity of produced antibodies against VP4 synthitic peptides.	102
4.56	Antisera IgM&IgG obtained from different groups immunized with synthetic peptides corresponding to VP1.	103
4.57	Antisera IgM&IgG obtained from different groups immunized with synthetic peptides corresponding to VP4.	104
4.58	Antisera IgM&IgGobtained from two groups immunized with synthetic peptides corresponding to VP2.	105
4.59	Representative result of antisera IgM obtained from two groups immunized with synthetic peptides corresponding to VP1.	107
4.60	Representative result of antisera IgG obtained from two groups immunized with synthetic peptides corresponding to VP4.	108
4.61	Representative result of antisera IgM and IgG obtained from two groups immunized with synthetic peptides corresponding to VP2 as single agent or combined with peptide corresponding VP1 and VP4.	109
4.62	Representative result of antisera IgG obtained from groups immunized with synthetic peptides or their corresponding proteins.	110
4.63	Inhibition of HRV-74 viral infectivity.	112
4.64	Inhibition of HRV-15 viral infectivity.	113
4.65	Inhibition of HRV-16 viral infectivity.	114
4.66	Inhibition of HRV-44 viral infectivity.	115

4.67 Inhibition of HRV-51 viral infectivity. 116

4.68 Inhibition of HRV-54 viral infectivity. 117

4.69 Inhibition of HRV-54 viral infectivity. 118

4.70 Inhibition of HRV-55 viral infectivity. 119

4.71 Inhibition of HRV-72 viral infectivity. 120

4.72 Inhibition of HRV-76 viral infectivity. 121

4.73 Inhibition of HRV-89 viral infectivity. 122

LIST OF ABBREVIATIONS

+ssRNA	Positive sense single-stranded RNA
Aa	Amino acid
Ag	Antigen
Amp	Ampicillin
ATCC	American Type Culture Collection
BLAST	Basic Linear Alignment Search Tool
bp	Base pair
BSA	Bovine serum albumin
C-terminus	Carboxy terminus
CDC	Centers for disease control and prevention
cDNA	Complementary DNA
CFA	Complete Freund's adjuvant
COPD	Chronic obstructive pulmonary disease
CPE	Cytopathic effect
D1-D5	Extracellular Ig-like domains
DAA-I	Des-aspartate-angiotensin I
DNA	Deoxyribonucleic acid
dH_2O	Distilled water
dNTPs	Deoxynucleotide triphosphate
dsDNA	Double strand DNA
ELISA	Enzyme-linked irnmunoabsorbent assay
EMEM	(DJOH¶V0LQLPXP(VVHQWLDO0HGLXP
Fab	Fragment antigen-binding
FBS	Foetal bovine serum
FDA	Food and Drug Administration
g	Gram
GC	Guanine-Cytosine
Gp	Envelope glycoprotein

H	Hour
H&L	Heavy and Light chains
H_2SO_4	Sulfuric acid
HA	Hemagglutinin (influenza viruses)
His-tag	Histidine residues Tag
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase (enzyme)
HRV	Human rhinovirus
ICAM-1	Intercellular adhesion molecule 1
ICTV	International Committee for Taxonomy of Viruses
IFA	Incomplete Freund's adjuvant
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPTG	Isopropyl-P-d-thiogalactopyranosi
Kb	Kilobase
kDa	Kilodalton
l	Litre
L-15	Leibovitz's L-15 Medium
LB	Luria Bertani
LDLR	Low-density lipoprotein receptor
LFA-1	Lymphocyte function-associated antigen 1
LRI	Lower respiratory infections
LRT	Lower respiratory tracts
M	Molar
mAb	Monoclonal antibody
MM	Millimolar

MEM	Minimum essential medium
min	Minute
mm	Millimeter
MOI	Multiplicity of infection
mRNA	Messenger ribonucleic acid
MW	Moleculer weight
N-terminus	Amino terminus
NCBI	National Centre for Biotechnology Information
NCR	Non-coding region
NdeI	Restriction enzyme sites
Ng	Nanogram
NIm	Neutralizing immunogenic site
NJ	Neighbour-joining
Nm	Nanometer
OD	Optical density
ORF	Open reading frame
qPCR	Quantitative real-time polymerase chain reaction
PAGE	Polyaccrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate-buffered saline and Tween 20
PVDF	Polyvinyli-dine difluoride
RDRP	RNA-dependent RNA polymerase
RG	Rabbit group
RNA	Ribonucleic acid
Rpm	Revolutions per minute
RT	Room temperature
RT-PCR	Reverse transcription polymerase chain reaction
RV	Rhinovirus
RV-A	Rhinovirus group A
RV-B	Rhinovirus group B

RV-C	Rhinovirus group C
RVs	Rhinoviruses
s	Second
SC	Subcutaneous
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SVDV	Swine vesicular disease virus
TBE	Tris-Borate-EDTA
TBS	Tris Buffered saline
TCID	Tissue culture infectious dose
TMB	• -tetramethylbenzidine
URT	Upper respiratory tracts
UTR	Untranslated region
V	Volt
V	Volume
VLDL	Very low-density lipoprotein
VRIs	Viral respiratory infections
VP	Virus protein
VPg	Viral priming protein
XhoI	Restriction enzyme sites

CHAPTER 1

GENERAL INTRODUCTION

Rhinoviruses (RVs) represent the most important cause of common cold and are well recognised as causative agents for the self-limiting disease of the respiratory tract. The use of advanced diagnostic methods in a wide variety of studies has demonstrated that RVs arealso associated with severity of respiratory symptoms. According to several reports, RVs are responsible for about 75% of acute asthma exacerbations and chronic obstructive pulmonary disease (COPD) in both children and adults(Mallia *et al*., 2011; Kameel & Steve, 2014). The number of cases is growing globally in each consecutive year which increases the burden of providing care worldwide (Peter, 2014). RVs are also implicated in more severe disease manifestations such as pneumonia (Broberg *et al*., 2011), acute bronchiolitis in young children (Renwick *et al.,* 2007), croup (Choi *et al.,* 2006) and otitis media (Chantzi, *et al.,* 2006). To date, exacerbation of these diseases has been poorly responsive to the current therapies.

RVs, which were formerly known as human rhinoviruses,were first isolated by Pelon *et al.* and Price in 1956 (Brooks *et al*., 2010). RVs represent a large number ofsmall non-enveloped viruses of about 28 to 30 nm in diameter within the genus *Enterovirus* of the *Picornaviridae* family (Knowles *et al.,* 2012).The viral genome is positive sense single-stranded RNA (+ssRNA) of approximately 7,200 bases (Turner & Lee, 2009). Within 50 years since their discovery, RVs have been divided into three groups, RV-A, -B and -C, with the latter, RV-C, whichonly reported in 2007 (Lau *et al*., 2007). There are more than 100 types of RV-A and B, while the discovery of new RV-C still continues (Simmonds *et al.,* 2010; McIntyre *et al.,* 2013).

The viral capsid, which surrounds the genomic RNA, is composed of 60 identical copies each of four structural proteins, designated as VP1, VP2, VP3 and VP4. The three larger proteins (VP1, VP2, and VP3) are exposed on the capsid surface and account for the virus' antigenic diversity, while the smallest one (VP4) lies at the interface between the capsid and the viral genome. The exposed proteins (VP1-3) have the same overall structural conformation, an eight-stranded antiparallel ß-barrel and without any remarkable sequence homology. C-termini of the exposed proteins are located on the surface of the virion, while amino (N) termini are in the interior. Among the four capsid proteins, VP1 is the largest and the most exposed, and it serves as the site of attachment to the cell surface receptors (Rossmann *et al.,* 1985; Jacobs *et al.,* 2013). The surface of the RVs capsid contains neutralization antigenic and host cell binding sites. The latter allows the virus to start its replication cycle by attaching to the host cell receptors (Rossmann *et al.,* 1994).

The N-terminus of VP1 and VP4 in several closely related *Picornaviruses* has been suggested to be externalized during the uncoating process. Together, they allow the viral particle to interact directly with the host cell by shaping a pore in the cell membrane, through which the viral RNA is released to the cytoplasm (Seechurn *et al*., 1990; Danthi *et al*., 2003; Tuthill *et al*., 2006; Davis *et al*., 2008).

The RV variants are also divided based on their receptor into major and minor groups. The major receptor group (100% of RV-B and 85% of RV-A) uses ICAM-1 for cell entry, while the minor group binds the low-density lipoprotein(LDL) receptor family including the LDL receptor itself, the very low-density lipoprotein (VLDL) receptor and the LDL receptor-related protein. In addition to ICAM-1, some types of the major group can use heparan sulphate as an additional receptor (Fuchs & Blaas, 2010). RV-C receptor is still not known, while at least one RV-C isolate (HRV-C15) utilizes a cellular receptor other than ICAM-1 or LDL (Bochkov *et al*., 2011).

Due to its transmission, avoiding RV infections is nearly impossible. Beside direct contact, millions of viral particles are transmitted via hundreds of droplets that can be released in a single sneeze, cough, or exhale during conversation. Although aerosol droplets travel only short distances (1-2 meters) before settlings on surfaces, viruses can remain infectious for a relatively long time (La Rosa *et al.*, 2013). Under experimental conditions, RVs will survive in an indoor environment for up to hours and days (Hendley *et al.*, 1973).

To date, there have been no effective and approved antiviral therapies for either the prevention or treatment of diseases caused by RVs infections. Several factors such as the large number of RVs serotypes with hypervariable sequence, the lack of animal model and the rapid emergence of new strains have hindered the progression of vaccine development. Meanwhile, many molecular epidemiological studies of RVs conducted in different regions have revealed that there are no predominant circulating serotypes which could be considered for vaccine development (Chan *et al.*, 2012; Rahamat-Langendoen *et al.*, 2013; Miller & Mackay, 2013; Etemadi *et al.*, 2013). However, as a group, RV-A is the most predominant species, and this is followed by the newly discovered group "RV-C", whereas RV-B is the least frequently detected species. Due to these facts, vaccines conventionally designed to generate neutralising antibodies are unlikely to provide sufficient and overall protection to frequent infections which occur throughout life. With the high RV burden which is poorly responsive to the current therapies, alternative approaches to overcome their infections are therefore needed.

Eliciting cross-neutralizing antibodies has been considered the words of interest in the search for effective RV vaccines. Capsid proteins (VP1-4) or antigenic peptides corresponding to one of them have been claimed to induce cross-neutralising antibodies against different RV strains (McCray & Werner, 1987; Edlmayr *et al*.,2011).

In the current study, alternative strategies were applied in an attempt to design a broad-spectrum RV vaccine based on the reverse approach. In the era of genomics, the starting point of designing an ideal vaccine against RVs could be from the available information on their genomes. Recently, the full-length genome sequences of all RV-A and RV-B serotyped strains have been reported (Palmenberg *et al*., 2009). This is a major step forward in the path of RVs vaccination. The reverse approach to vaccine development takes advantage of the pathogen's genome sequence. For instance, such approach has been used to develop a broadly protective vaccine against serogroup B *Neisseria meningitidis* byidentifying five proteins that are conserved across the strains (Please refer toGiuliani *et al*., 2006; Toneatto *et al*.,

REFERENCES

Adams, M.J., King, A.M.Q. and Carstens, E.B. (**2013**).Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2013). Archives of Virology 158: 2023–2030. http://www.ictvonline.org

American Lung Association.(**2012**). Trends in asthma morbidity and mortality. Retrieved from http://www.lung.org/finding-cures/our-research/trend-reports/asthma-trend-report.pdf

Anga, Lay-Teng, Ling-Yin Tana, Vincent T. Chowb, Meng-Kwoon Sim .(**2012**). Des-aspartate-angiotensin I exerts antiviral effects and attenuates ICAM-1 formation in rhinovirus-infected epithelial cells. *Eurp J Pharm*, Volume 683, Issues 1–3, Pages 310–315

Anzueto A, Niederman MS (**2003)**. Diagnosis and treatment of rhinovirus respiratory infections.*Chest*, 123:1664-1672

Arruda E, Pitkaranta A, Witek TJ Jr., Doyle CA, Hayden FG (**1997)**. Frequency and natural history of rhinovirus infections in adults during autumn. J Clin Microbiol;35:2864-8

Bartlett NW, Walton RP, Edwards MR, et al (**2008)**. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med; **14**: 199–204.

Basta, H. A., Sgro, J. Y., & Palmenberg, A. C. (**2014**). Modeling of the human rhinovirus C capsid suggests a novel topography with insights on receptor preference and immunogenicity. *Virology*, *448*, 176-184.

Belser JA, Rota PA, Tumpey TM. (**2013)**. Ocular tropism of respiratory viruses. Microbiol.Mol. Biol. Rev. **77**:144–156.

Belshe, R. B., Gruber, W. C., Mendelman, P. M., Cho, I., Reisinger, K., Block, S. L., ... & Wolff, M. (**2000**). Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *The Journal of pediatrics*, *136*(2), 168-175.

Benen, T. D., Tonks, P., Kliche, A., Kapzan, R., Heeney, J. L., & Wagner, R. (**2014**).Development and immunological assessment of VLP-based immunogens exposing the membrane-proximal region of the HIV-1 gp41 protein. *Journal of biomedical science*, *21*(1), 1-14.

Berchoff, R. P. (**1987)**. Picornaviruses at the Molecular Level, p. 197-215. In R. P. Berchoff (ed.), The Molecular Basis of Viral Replication. Plenum Press, New York.

Bischoff WE. (**2010)**. Transmission route of rhinovirus type 39 in a monodispersed airborne aerosol. Infect. Control Hosp. Epidemiol. 31: 857–859

Blomqvist S, Roivainen M, Puhakka T, Kleemola M, Hovi T. (**2002)**. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. J Med Virol;66:263–268

Blomqvist, Soile.,(**2004)** Epidemiology of human rhinoviruses, University of Helsinki, Finland, thesis,.

Bloom, B., Cohen, R. A., & Freeman, G. (**2009**). Summary health statistics for US children: National Health Interview Survey, 2008. *Vital and health statistics. Series 10, Data from the National Health Survey*, (244), 1-81.

Bloom, B., Cohen, R. A., & Freeman, G. (**2012**). Summary health statistics for us Children: national health interview survey, 2011. *Vital and health statistics. Series 10, Data from the National Health Survey*, (254), 1-88.

Broberg, E., Niemelä, J., Lahti, E., Hyypiä, T., Ruuskanen, O., Waris, M.(**2011**).Human rhinovirus C-Associated severe pneumonia in a neonate *Journal of Clinical Virology*, 51 (1), pp. 79-82.

Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A., & Mietzner, T. A. (**2010**). Jawetz, elnick, & Adelberg's edical icrobiology, The cGraw-Hill Companies

dDOÑNDQ M., Bochkov, Y. A., Kreiner-Møller, E., Bønnelykke, K., Stein, M. M., Du, G., ...& Ober, C. (**2013**). Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *New England Journal of Medicine*, *368*(15), 1398-1407.

Callahan, P. L., Mizutani, S., & Colonno, R. J. (**1985**). Molecular cloning and complete sequence determination of RNA genome of human rhinovirus type 14.*Proceedings of the National Academy of Sciences*, *82*(3), 732-736.

Cardoso, R. M., Zwick, M. B., Stanfield, R. L., Kunert, R., Binley, J. M., Katinger, H., ...& Wilson, I. A. (**2005**). Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly conserved fusion-associated motif in gp41. *Immunity*, *22*(2), 163-173.

Carey, B.S., Barclay, W.S., Russell, S.M., Tyrrell, D.A., (**1992).**The specificity of antibodies induced by infection with rhinovirus type 2. *J. Med. Virol*.; 36, 251–258.

Casasnovas, J. M. & Springer, T. A. (**1995**).Kinetics and thermodynamics of virus binding to receptor. Journal of Biological Chemistry 270, 13216–24.

Cate, T. R., Rossen, R. D., Douglas, R. G., Jr., Butler, W. T., and Couch, R. B., (**1966**). The role of nasal secretion and serum antibody in the rhinovirus common cold. Amer. J. Epidemiol. 84:352. 20.

Centersfor Disease Control andPrevention, National Center for Health Statistics.(**2014**).*Asthma*. Retrieved from http://www.cdc.gov/nchs/fastats /asthma.htm

Chan, Y. F., Jafar, F. L., Nathan, A. M., de Bruyne, J. A., Hassan, A., Nor'e, S. S., ...& Sam, I. (**2012**). Diverse human rhinoviruses A and C from children with respiratory infections in Kuala Lumpur, Malaysia. *Journal of Infection*, *64*(6), 633-636.

Chantzi, F.M., Papadopoulos, N.G., Bairamis, T., Tsiakou, M., Bournousouzis, N., Constantopoulos, A.G., Liapi, G., Kafetzis, D.A.(**2006**). Human rhinoviruses in otitis media with effusion *Pediatric Allergy and Immunology*, 17 (7), pp. 514-518.

Chapple, P. J., Head, B., and Tyrrell, D. A. J.,(**1967**).A complement fixing antigen from an M rhinovirus. Arch. Gesamte. Virusforsch.21, 123.

Choi, E.H., Lee, H.J., Kim, S.J., Eun, B.W., Kim, N.H., Lee, J.A., Lee, J.H., Sung, J.Y.(**2006**).The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. Clinical Infectious Diseases, 43 (5), pp. 585-592.

Coffman, R. L., Sher, A., & Seder, R. A. (**2010**). Vaccine adjuvants: putting innate immunity to work. *Immunity*, *33*(4), 492-503.

Colonno RJ, Callahan PL, Leippe DM, et al.(**1989)**. Inhibition of rhinovirus attachment by neutralizing monoclonal antibodies and their Fab fragments. J Virol ; 63: 36–42.

Colonno RJ, Callahan PL, Long WJ.(**1986).** Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses. J Virol;57:7–12.

Colonno RJ, Condra JH, Mizutani S, Callahan PL, Davies ME, Murcko MA. (**1988)**. Evidence for the direct involvement of the rhinovirus canyon in receptor binding. Proc Natl Acad Sci USA; 85: 5449–5453.

Comas, I., Chakravartti, J., Small, P. M., Galagan, J., Niemann, S., Kremer, K., ...& Gagneux, S. (**2010**). Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. Nature genetics, 42(6), 498-503.

Conant, R.M., Hamparian, V.V., (**1968)**. Rhinoviruses: Basis for a numbering system. II. Serologic characterization of prototype strains. J. Immunol. 100, 114–119.

Contorni, M. Morandi, A. Bartalesi, V. Cinotti, D. Mannucci, F. Titta, E. Ovidi, J. A. Welsch, D. Granoff, R. Rappuoli, and M. Pizza. **(2006)**. A universal vaccine for serogroup B meningococcus. Proc. Natl. Acad. Sci. USA 103**:**10834-10839.

Cox, D. W., & Le Souëf, P. N. (**2014**).Rhinovirus and the developing lung.*Paediatric respiratory reviews*.

Cruz, A. A. (**2007**). Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. J. Bousquet, & N. G. Khaltaev (Eds.).World Health Organization.

'\$OHVVLR D, Peterson J, Dick C, Dick E. (**1976)**. Transmission of experimental rhinovirus colds in volunteer married couples. J. Infect. Dis. 133:28 –36**.**

Danthi, P., M. Tosteson, Q. H. Li, and M. Chow. (**2003)**. Genome delivery and ion channel properties are altered in VP4 mutants of poliovirus. J. Virol.77**:**5266-5274.

Davis, M. P., Bottley, G., Beales, L. P., Killington, R. A., Rowlands, D. J., & Tuthill, T. J. (**2008**). Recombinant VP4 of human rhinovirus induces permeability in model membranes. *Journal of virology*, *82*(8), 4169-4174.

De Palma, A. M.; Vliegen, I.; De Clercq, E.; Neyts, J. (**2008).**Selective inhibitors of picornavirus replication. ed. Res. Rev., 28 (6), 823−884.

Del Vecchio, A. M., Branigan, P. J., Barnathan, E. S., Flavin, S. K., Silkoff, P. E., & Turner, R. B. (**2014**). Utility of animal and in vivo experimental infection of humans with rhinoviruses in the development of therapeutic agents for viral exacerbations of asthma and chronic obstructive pulmonary disease.*Pulmonary pharmacology & therapeutics*.

Desjardins, P., Hansen, J. B. & Allen, M. (**2009**).Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer.J Vis Exp (33), 1610.

Desmond W. Cox, Joelene Bizzintino, Giovanni Ferrari, Siew Kim Khoo, Guicheng Zhang, Siobhan Whelan, Wai Ming Lee,Yury A. Bochkov, Gary C. Geelhoed, Jack Goldblatt, James E. Gern, Ingrid A. Laing, and Peter N. Le Souëf.**(2013)** Human Rhinovirus Species C Infection in Young Children with Acute Wheeze Is Associated with Increased Acute Respiratory Hospital Admissions. American Journal of Respiratory and Critical Care Medicine 188:11, 1358-1364

DiazGranados, C. A., Denis, M., & Plotkin, S. (**2012**). Seasonal influenza vaccine efficacy and its determinants in children and non-elderly adults: a systematic review with meta-analyses of controlled trials. *Vaccine*, *31*(1), 49-57.

Dick, EC., LC Jennings, KA Mink, CD Wartgow, SL Inhorn Aerosol transmission of rhinovirus coldsJ Infect Dis, 156 (**1987**), pp. 442–448

Douglas, R. G., Jr., Rossen, R. D., Butler, W. T., and Couch, R. B.,(**1967**).Rhinovirus neutralizing antibody in tears, parotid saliva, nasal secretions and serum. J. Immunol. 99,297.

Edlmayr J, Niespodziana K, Popow-Kraupp T, et al **(**.2011). Antibodies induced with recombinant VP1 from human rhinovirus exhibit cross-neutralisation.*Eur Respir J* ; 37: 44–52.

Emuzyte R, Firantiene R, Petraityte R, Sasnauskas K (**2009**) Human rhinoviruses, allergy, and asthma: a clinical approach. Medicina (Kaunas) 45: 839-847.

Ekiert, D. C., Bhabha, G., Elsliger, M. A., Friesen, R. H., Jongeneelen, M., Throsby, M., ...& Wilson, I. A. (**2009**). Antibody recognition of a highly conserved influenza virus epitope. *Science*, *324*(5924), 246-251.

Etemadi, M. R., Othman, N., Savolainen-Kopra, C., Sekawi, Z., Wahab, N., & Sann, L. M. (**2013**).Biodiversity and clinico-demographic characteristics of human rhinoviruses from hospitalized children with acute lower respiratory tract infections in Malaysia. *Journal of Clinical Virology*, *58*(4), 671-677.

Feil, S.C. ; S. Hamilton, G.Y. Krippnert, B. Lin, A. Luttick, D.B. McConnell.(**2012**).An orally available 3-ethoxybenzisoxazole capsid binder with clinical activity against human rhinovirus ACS Med Chem Lett, 3, pp. 303–307

Fang F, Yu M. (**2004)**. Viral receptor blockage by multivalent recombinant antibody fusion proteins:Inhibiting human rhinovirus (HRV) infection with CFY196. J Antimicrob Chemother;53:23–25.

Fling SP, Gregerson DS (**1986)**. Peptide and protein molecular weightdetermination by electrophoresis using a high-molarity tris buffersystem without urea. Anal Biochem; 155: 83–88.

Fry AM, Lu X, Olsen SJ, Chittaganpitch M, Sawatwong P, Chantra S, Baggett HC, Erdman D.**(2011)**. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. PLoS One **6**:e17780.

Fox, J. P. (**1976**). Is a rhinovirus vaccine possible? Am J Epidemiol 103, 345-54.

Fendrick, A. M., Monto, A. S., Nightengale, B., & Sarnes, M. (2003).The economic burden of non–influenza-related viral respiratory tract infection in the United States. *Archives of Internal Medicine*, *163*(4), 487-494.

Fox, J. P., Cooney, M. K., Hall, C. E. & Foy, H. M. (**1985**). Rhinoviruses in Seattle families, 1975-1979. Am J Epidemiol 122, 830-46.

Fuchs R, Blaas D (**2010**) Uncoating of human rhinoviruses. Rev Med Virol 20: 281–297.

Gagnon, Mylène Claude; Williams, Martin; Doucet, Alain And Beauregard, Marc. (**2000).** Replacement Of Tyr62 By Trp In The Designer Protein Milk Bundle-1 results in significant improvement of conformational stability. *FEBS Letters*, November, vol. 484, no. 2, p. 144-148.

Garriga, D., Pickl-Herk, A., Luque, D., Wruss, J., Castón, J. R., Blaas, D., and Verdaguer, N.(**2012**). Insights into minor group rhinovirus uncoating: the X-ray structure of the HRV2 empty capsid. *PLoS pathogens* 8, e1002473

Gilbert, S. C. (**2012**). Tæcellæinducing vaccines–what's the future. *Immunology*,*135*(1), 19-26.

Giuliani MM, Adu-Bobie J, Comanducci M, Aricò B, Savino S, Santini L, Brunelli B, Bambini S, Biolchi A, Capecchi B, Cartocci E, Ciucchi L, Di Marcello F, Ferlicca F, Galli B, Luzzi E, Masignani V, Serruto D, Veggi D, Contorni M, Morandi M, Bartalesi A, Cinotti V, Mannucci D, Titta F, Ovidi E, Welsch JA, Granoff D, Rappuoli R, Pizza M. (**2006**). A universal vaccine for serogroup B meningococcus. Proc. Natl. Acad. Sci. U. S. A. 103:10834–10839.

Glanville N, Mclean GR, Guy B, Lecouturier V, Berry C, et al. (**2013**) Cross-Serotype Immunity Induced by Immunization with a Conserved Rhinovirus Capsid Protein. PLoS Pathog 9(9): e1003669. doi:10.1371/journal.ppat.1003669

Goodman, A. L., C. Epp, D. Moss, A. A. Holder, J. M. Wilson, C. Gao, A. Long, E. J. Remarque, A. W. Thomas, V. Ammendola, et al. (**2010)**. New candidate vaccines against blood-stage Plasmodium falciparum malaria: prime-boost immunization regimes incorporating human and simian adenoviral vectors and poxviral vectors expressing an optimized antigen based on merozoite surface protein 1. Infect. Immun. 78: 4601–4612.

Graat J, Schouten E, Heijnen M, Kok F, Pallast EGM, de Greeff S, Dorigo-ZetsmaJ**. (2003)**. A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection. J. Clin. Epidemiol.56:1218 –1223.

Greve, J. M., Davis, G., Meyer, A. M., Forte, C. P., Yost, S. C., Marlor, C. W., Kamarck, M. E., and McClelland, A. (**1989**) The major human rhinovirus receptor is ICAM-1. Cell 56, 839–847

Guilbert, T. W., Singh, A. M., Danov, Z., Evans, M. D., Jackson, D. J., Burton, R., ...& Lemanske Jr, R. F. (**2011**). Decreased lung function after preschool wheezing rhinovirus illnesses in children at risk to develop asthma. *Journal of Allergy and Clinical Immunology*, *128*(3), 532-538.

Gwaltney Jr., J.M., (**1985)**. Virology and immunology of the common cold.Rhinol. 23, 265–271.

Gwaltney, J. M., Jr., Hendley, J. O., Simon, G. & Jordan, W. S., Jr. (**1967**).Rhinovirus infections in an industrial population. II. Characteristics of illness and antibody response. Jama 202, 494-500.

Gwaltney Jr, PB Moskalski, JO Hendley. (**1978**). Hand-to-hand transmission of rhinovirus coldsAnn Intern Med, 88, pp. 463–467

Hamparian VV, Colonno RJ, Cooney MK, Dick EC, Gwaltney JM Jr, et al. (**1987**) A collaborative report: *Rhinoviruses*-extension of the numbering system from 89 to 100. Virology 159(1): 191–192.

Han, J. H., Choi, Y. S., Kim, W. J., Jeon, Y. H., Lee, S. K., Lee, B. J., & Ryu, K. S. (**2010**). Codon optimization enhances protein expression of human peptide deformylase in E. coli. *Protein expression and purification*,*70*(2), 224-230.

Hao W, Bernard K, Patel N, Ulbrandt N, Feng H, Svabek C, Wilson S, Stracener C, Wang K, Suzich J, Blair W, Zhu Q. (**2012)**. Infection and propagation of human rhinovirus C in human airway epithelial cells. J. Virol.86:13524–13532.

Harris, J. R., & Racaniello, V. R. (**2005**). Amino acid changes in proteins 2B and 3A mediate rhinovirus type 39 growth in mouse cells. *Journal of virology*,*79*(9), 5363-5373.

Hastings GZ, Francis MJ, Rowlands DJ, Chain BM (**1993**) Epitope analysis of the T cell response to a complex antigen: proliferative responses to human rhinovirus capsids. Eur J Immunol 23: 2300–2305. doi: 10.1002/eji.1830230937

Hastings, G. Z., Speller, S. A., & Francis, M. J. (**1990**). Neutralizing antibodies to human rhinovirus produced in laboratory animals and humans that recognize a linear sequence from VP2. *Journal of general virology*, *71*(12), 3055-3059.

Hayden, F. G.; Herrington, D. T.; Coats, T. L.; Kim, K.; Cooper, E. C.; Villano, S. A.; Liu, S.; Hudson, S.; Pevear, D. C.; Collett, M.; McKinlay, M. (**2003a).**Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: Results of 2 doubleblind, randomized, placebo-controlled trials. Clin. Infect. Dis., 36 (12), 1523−1532.

Hayden, F. G., Gwaltney, J. M. & Colonno, R. J. (**1988**).Modification of experimental rhinovirus colds by receptor blockade. Antiviral Research 9, 233–47

Hayden, F.G.; R.B. Turner, J.M. Gwaltney, K. Chi-Burris, M. Gersten, P. Hsyu, A.K. Patick, G.J. Smith 3rd, L.S. Zalman. (**2003b**). Phase II, randomized, double-blind, placebo-controlled studies of ruprintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers Antimicrobial agents and chemotherapy, 47, pp. 3907–3916

Heikkinen T, Jarvinen A **(2003)** The common cold. Lancet 361: 51–59.

Hendley J, Wenzel R, Gwaltney JM, Jr**. (1973)**. Transmission of rhinovirus colds by self-inoculation. N. Engl. J. Med. 288:1361–1364

Hennessy TW, Ritter T, Holman RC, Bruden DL, Yorita KL, Bulkow L, Cheek JE, Singleton RJ, Smith J.**(2008)**. The relationship between in-home water service and the risk of respiratory tract, skin, and gastrointestinal tract infections among rural Alaska natives. Am. J. Public Health 98:2072–2078.

Hewat, E. A., & Blaas, D. (**1996**). Structure of a neutralizing antibody bound bivalently to human rhinovirus 2. *The EMBO journal*, *15*(7), 1515

Hillaire, M. L., Osterhaus, A. D., & Rimmelzwaan, G. F. (**2011**).Induction of virus-specific cytotoxic T lymphocytes as a basis for the development of broadly protective influenza vaccines. *BioMed Research International*, *2011*.

Hofer, F., Gruenberger, M., Kowalski, H., Machat, H., Huettinger, M., Kuechler, E., Blaas, D., (**1994)**. Members of the low density lipoprotein receptor family mediate cell entry of a minor-group common cold virus. Proc. Natl. Acad. Sci. USA. 91, 1839–1842.

Hu, Wei-Gang, Junfei Yin, Damon Chau, Charles Chen Hu, Dustin Lillico, Justin Yu, Laurel M. Negrych, and John W.Cherwonogrodzky. (**2012**)."Conformation-dependent high-affinity potent ricin-neutralizing monoclonal antibodies." *BioMed research international* 2013

Hurst, J. R., Donaldson, G. C., Quint, J. K., Goldring, J. J., Baghai-Ravary, R., & Wedzicha, J. A. (**2009**). Temporal clustering of exacerbations in chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*, *179*(5),369-374.

Hughes, P. J., North, C., Jellis, C. H., Minor, P. D., & Stanway, G. (**1988**). The nucleotide sequence of human rhinovirus 1B: molecular relationships within the rhinovirus genus. *J Gen Virol*, *69*(Pt 1), 49-58.

Iwane MK,Prill MM, Lu X, Miller EK, Edwards KM, Hall CB, Griffin MR, Staat MA, Anderson LJ, Williams JV, Weinberg GA, Ali A, Szilagyi PG, Zhu Y, Erdman DD. (**2011)**. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. J. Infect. Dis. 204:1702–1710.

Iwasaki, J., Smith, W. A., Khoo, S. K., Bizzintino, J., Zhang, G., Cox, D. W., ... & Hales, B. J. (**2014**). Comparison of rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus infection. *Journal of Allergy and Clinical Immunology*.

Jackson DJ, Gangnon RE, Evans MD, *et al* . (**2008)** Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med;178:667–72.

Jacobs SE, Lamson DM, George KS, Walsh TJ.(**2013)** Human Rhinoviruses.Clin.Microbiol. Rev.;26(1):135–162.

Jang Y. J., Wang J. H., Kim J. S., Kwon H. J., Yeo N. K., Lee B. J. (**2009**). Levocetirizine inhibits rhinovirus-induced ICAM-1 and cytokine expression and viral replication in airway epithelial cells. Antiviral Res. 81, 226–233.

Jimenez-Clavero, M. A., Douglas, A., Lavery, T., Garcia-Ranea, J. A., & Ley, V. (**2000**). Immune recognition of swine vesicular disease virus structural proteins: novel antigenic regions that are not exposed in the capsid. *Virology*,*270*(1), 76-83.

Johnston, S. L., Pattemore, P. K., Sanderson, G., Smith, S., Lampe, F., Josephs, L., & Holgate, S. T. (**1995**). Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *Bmj*, *310*(6989), 1225-1229.

Kameel, M., & Steve, S. (**2014**). The spatial epidemiology of asthma: a chronic non-communicable disease and a neglected epidemic.Journal of Allergy and Asthma.,ISSN 2054-9873.,V: 1, Art: 2

Kapikian AZ, Conant RM, Hamparian VV, Chanock RM, Chapple PJ, et al. (**1967**). *Rhinoviruses*: a numbering system. Nature 213: 761–762.

Katpally U, Fu TM, Freed DC, et al. (**2009)**. Antibodies to the buried N terminus of rhinovirus VP4 exhibit cross-serotypic neutralization. J Virol ; 83:7040–7048.

Kapusinszky, B., Phan, T.G., Kapoor, A. and Delwart, E. (**2012**). Genetic diversity of the genus *Cosavirus* in the family *Picornaviridae*: a new species, recombination, and 26 new genotypes. PLoS One. 7(5):e36685. Epub 2012 May 16.

Kelly, J.T., Busse, W.W. (**2008**). Host immune responses to rhinovirus: Mechanisms in asthma, *Journal of Allergy and Clinical Immunology*, 122 (4), pp. 671-

Khan AM, Miotto O, Heiny AT, Salmon J, Srinivasan KN, Nascimento EJ, Marques ET Jr, Brusic V, Tan TW, August JT. (**2006)**.A systematic bioinformatics approach for selection of epitope-based vaccine targets.*Cell Immunol*, 244:141-7

Khan AM, Miotto O, *et al.* (**2008**) Conservation and variability of dengue virus proteins: implications for vaccine design. PLoS Negl Trop Dis 2: e272

Khor, C.S., I.C. Sam, P.S. Hooi, K.F. Quek, Y.F. (**2012).** Chan Epidemiology and seasonality of respiratory viral infections in hospitalized children in Kuala Lumpur, Malaysia: a retrospective study of 27 years BMC Pediatr., 20, p. 32

Kiener, T. K., Jia, Q., Meng, T., Chow, V. T. K., & Kwang, J. (**2014**). A Novel Universal Neutralizing Monoclonal Antibody against Enterovirus 71 That Targets the Highly Conserved "Knob" Region of VP3 Protein. *PLoS neglected tropical diseases*, *8*(5), e2895.

Kistler, A. L., Webster, D. R., Rouskin, S., Magrini, V., Credle, J. J., Schnurr, D. P., ...& DeRisi, J. L. (**2007**). Genome-wide diversity and selective pressure in the human rhinovirus. *Virology journal*, *4*(1), 40.

Kiyota, N., Kobayashi, M., Tsukagoshi, H., Ryo, A., Harada, S., Kusaka, T., ...& Kimura, H. (2014). Genetic analysis of human rhinovirus species A to C detected in patients with acute respiratory infection in Kumamoto prefecture, Japan 2011–2012. *Infection, Genetics and Evolution*, *21*, 90-102.

Knopf, H.L., Perkins, J.C., Bertran, D.M., Kapikian, A.Z., Chanock, R.M., (**1970)**. Analysis of the neutralizing activity in nasal wash and serum following intranasal vaccinaion with inactivated type 13 rhinovirus. J. Immunol. 104, 566–573

Knowles, N.J., Hovi, T, Hyypiä, T., King, A.M.Q., Lindberg, A.M., Pallansch, M.A., Palmenberg, A.C., Simmonds, P., Skern, T., Stanway, G., Yamashita, T. and Zell, R. (**2012**).Picornaviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Ed: King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. San Diego: Elsevier, pp 855-880.

Kolatkar, P. R., Bella, J., Olson, N. H., Bator, C. M., Baker, T. S., & Rossmann, M. G. (**1999**).Structural studies of two rhinovirus serotypes complexed with fragments of their cellular receptor. *The EMBO journal*, *18*(22), 6249-6259.

Kusel, M. M., Kebadze, T., Johnston, S. L., Holt, P. G., & Sly, P. D. (**2012**). Febrile respiratory illnesses in infancy and atopy are risk factors for persistent asthma and wheeze. *European Respiratory Journal*, *39*(4), 876-882.

La Rosa G, Fratini M, Della Libera S, Iaconelli M, Muscillo M.. (**2013**). Viral infections acquired indoors through airborne, droplet or contact transmission; Ann Ist Super Sanita 49(2):124-32.

Laine, P., Blomqvist, S., Savolainen, C., Andries, K., & Hovi, T. (**2006**). Alignment of capsid protein VP1 sequences of all human rhinovirus prototype strains: conserved motifs and functional domains. *Journal of general virology*,*87*(1), 129-138..

Laine, P., Savolainen, C., Blomqvist, S., & Hovi, T. (**2005**). Phylogenetic analysis of human rhinovirus capsid protein VP1 and 2A protease coding sequences confirms shared genus-like relationships with human enteroviruses.Journal of general virology, 86(3), 697-706.

Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St George K, Briese T, Lipkin WI. (**2006)**. MassTag polymerase-chainreaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004-2005. J. Infect. Dis. 194:1398 –1402.

Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, et al. (**2007**) Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 45: 3655–3664. doi: 10.1128/JCM.01254-07

Lau SKP, Yip CCY, Lin AWC, Lee RA, So LY, Lau YL, et al. (**2009).** Clinical and molecular epidemiology of a novel rhinovirus species, human rhinovirus C. J Infect Dis. ;200:1096–103

Lauinger, I. L., Bible, J. M., Halligan, E. P., Bangalore, H., Tosas, O., Aarons, E. J.,& Tong, C. Y. (**2013**). Patient characteristics and severity of human rhinovirus infections in children. *Journal of Clinical Virology*, *58*(1), 216-220.

Lee MS, Hseu YC, Lai GH, Chang WT, Chen HJ, Huang CH, et al. (**2011**). High yield expression in a recombinant E. coli of a codon optimized chicken anemia virus capsid protein VP1 useful for vaccine development. Microb Cell Fact; 10:56; PMID:21781331

Lee, W. M., Monroe, S. S., & Rueckert, R. R. (**1993**). Role of maturation cleavage in infectivity of picornaviruses: activation of an infectosome. *Journal of virology*, *67*(4), 2110-2122.

Lee, W. M., Wang, W., & Rueckert, R. R. (**1995**). Complete sequence of the RNA genome of human rhinovirus 16, a clinically useful common cold virus belonging to the ICAM-1 receptor group. *Virus genes*, *9*(2), 177-181.

Ledford, R. M., Patel, N. R., Demenczuk, T. M., Watanyar, A., Herbertz, T., Collett, M. S., & Pevear, D. C. (**2004**). VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. *Journal of virology*, *78*(7), 3663-3674.

Létourneau, S., Im, E. J., Mashishi, T., Brereton, C., Bridgeman, A., Yang, H., ...& Hanke, T. (**2011**). Correction: Design and Pre-Clinical Evaluation of a Universal HIV-1 Vaccine. *PloS one*, *6*(3).

Lewis JK, Bothner B, Smith TJ, Siuzdak G (**1998**) Antiviral agent blocks breathing of the common cold virus. Proc Natl Acad Sci U S A 95: 6774–6778.

Lewis, J. K., B. Bothner, T. J. Smith, and G. Siuzdak., (**1985).**Antiviral agent blocks breathing of the common cold virus. *Proc. Natl. Acad. Sci.* USA; 95:6774–6778.

Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, Poovorawan Y. (**2009**). High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease.*J Infect* , 59**:**115-121.

Lomax, N.B. & Yin, F.H.(**1989**).Evidence for the role of the P2 protein of human rhinovirus inits host range change. J. Virol. 63, 2396–2399.

Mäkelä, MJ., T Puhakka, O Ruuskanen *et al.*(**1998**).Viruses and bacteria in the etiology of the common cold. J Clin Microbiol, 36, pp. 539–542

Mallia P, Message SD, Gielen V, Contoli M, Gray K, et al. (**2011**) Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation. Am J Respir Crit Care Med 183: 734–74

McCray J, Werner G . (**1987).** Different rhinovirus serotypes neutralized by antipeptide antibodies. Nature ; 329: 736–738.

McIntyre, Chloé Leanne. (**2013).** The Epidemiology, Classification and Evolution of Human Rhinoviruses, The University of Edinburgh, Thesis,

McIntyre, C.L., Knowles, N.J. and Simmonds, P. (**2013**). Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. J. Gen. Virol. 94: 1791-1806.

McLean GR, Walton RP, Shetty S, Paktiawal N, Kebadze T, et al. (**2012**) Rhinovirus infections and immunisation induce cross-serotype reactive antibodies to VP1. Antiviral Res 95: 193–201. doi: 10.1016/j.antiviral.2012.06.006

McMurry, J. A., B. E. Johansson, and A. S. De Groot**.** (**2008)**. A call to cellular & humoral arms: enlisting cognate T cell help to develop broad-spectrum vaccines against influenza A. Hum. Vaccines **4:**148-157.

Menzella, Hugo G.(**2011**) "Comparison of two codon optimization strategies to enhance recombinant protein production in Escherichia coli." *Microb Cell Fact.*,10 15.

Miller, E. K., & Mackay, I. M. (**2013**). From sneeze to wheeze: What we know about rhinovirus Cs. *Journal of Clinical Virology*, *57*(4), 291-299

Mo, C., Yamagata, R., Pan, A., Reddy, J., Hazari, N., & Duke, G. (**2008**).Development of a high-throughput Alamar blue assay for the determination of influenza virus infectious dose, serum antivirus neutralization titer and virus phenotype. *Journal of virological methods*, *150*(1), 63-69.

Montero, M., van Houten, N. E., Wang, X., & Scott, J. K. (**2008**). The membrane-proximal external region of the human immunodeficiency virus type 1 envelope: dominant site of antibody neutralization and target for vaccine design. *Microbiology and molecular biology reviews*, *72*(1), 54-84.

Monto, A. S., Bryan, E. R. & Ohmit, S. (**1987**). Rhinovirus infections in Tecumseh, Michigan: frequency of illness and number of serotypes. J Infect Dis 156, 43-9

Monto AS, Ullman BM. (**1974).** Acute respiratory illness in an American community.The Tecumseh study.lAMA;227: 164-9

Mubareka, S., Louie, L., Wong, H., Granados, A., Chong, S., Luinstra, K., ...& Simor, A. (**2013**). Co-circulation of multiple genotypes of human rhinovirus during a large outbreak of respiratory illness in a veterans' long-term care home.*Journal of Clinical Virology*, *58*(2), 455-460.

Murphy, S. L., Xu, J. Q., & Kochanek, K. D. (**2013**). Deaths: final data for 2010. *National vital statistics reports*, *61*(4), 1-118.

Nicholson, K G., J Kent, V Hammersley, E Cancio.(**1997**). Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden BMJ, 315, pp. 1060–1064

Niespodziana, K., Cabauatan, C. R., Jackson, D. J., Gallerano, D., Trujillo-Torralbo, B., del Rosario, A., ...& Johnston, S. L. (**2014**). Rhinovirus-induced VP1-specific antibodies are group-specific and associated with severity of respiratory symptoms. *EBioMedicine*.

Niespodziana, K., Napora, K., Cabauatan, C., Focke-Tejkl, M., Keller, W., Niederberger, V., ...& Valenta, R. (**2012**). Misdirected antibody responses against an N-terminal epitope on human rhinovirus VP1 as explanation for recurrent RV infections. *The FASEB Journal*, *26*(3), 1001-1008.

Ohmit, S. E., Thompson, M. G., Petrie, J. G., Thaker, S. N., Jackson, M. L., Belongia, E. A., ...& Monto, A. S. (**2014**). Influenza vaccine effectiveness in the 2011–2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. *Clinical infectious diseases*, *58*(3), 319-327.

Owens RM, Gu X, Shin M, Springer TA, Jin MM. (**2010**).Engineering of single Ig superfamily domain of intercellular adhesion molecule 1 (ICAM-1) for native fold and function. J Biol Chem 285: 15906–15915. doi: 10.1074/jbc.M110.104349

Palmenberg AC, Spiro D, Kuzmickas R, et al.(**2009)**. Sequencing and analyses of all known human rhinovirus genomes reveals structure and evolution.Science ; 324: 55–59.

Palmenberg AC, Rathe JA, Liggett SB .(**2010)**. Analysis of the complete genome sequences of human rhinovirus. J Allergy Clin Immunol ; 125:1190–1199.

Papadopoulos, N. G., Bates, P. J., Bardin, P. G., Papi, A., Leir, S. H., Fraenkel, D. J., ...& Johnston, S. L. (**2000**). Rhinoviruses infect the lower airways. *Journal of Infectious Diseases*, *181*(6), 1875-1884.

Patick, A. K. (**2006).** Rhinovirus chemotherapy.Antiviral Res., 71 (2−3), 391−396.

Patick, A. K.; Brothers, M. A.; Maldonado, F.; Binford, S.; Maldonado, O.; Fuhrman, S.; Petersen, A.; Smith, G. J. 3rd; Zalman, L. S.; Burns-Naas, L. A.; Tran, J. Q. (**2005).** In vitro antiviral activity and singledose pharmacokinetics in humans of a novel, orally bioavailable inhibitor of human rhinovirus 3C protease.Antimicrob. Agents Chemother., 49 (6), 2267−2275

Pedersen, S. (**1984**). Escherichia coli ribosomes translate in vivo with variable rate. *The EMBO journal*, *3*(12), 2895.

Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypiä T**. (2008)**. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J. Infect. Dis. 197:382– 389.

Peter W. Heymann,. (**2014).** Developing Strategies to Treat Asthma Exacerbations Caused by Rhinovirus, EBioMedicine, Available online, ISSN 2352-3964,

Picornaviridae website. Available: http://www.picornaviridae.com/enteroviruses/enterovirus.htm. Accessed **2014** May 4.

Plotkin, S. A. (**2009**). Vaccines: the fourth century. *Clinical and Vaccine Immunology*, *16*(12), 1709-1719.

Puig, C., Fríguls, B., Gómez, M., García-Algar, Ó., Sunyer, J., & Vall, O. (**2010**). Relationship between lower respiratory tract infections in the first year of life and the development of asthma and wheezing in children. *Archivos de Bronconeumología (English Edition)*, *46*(10), 514-521.

Rahamat-Langendoen, J. C., RiezebosæBrilman, A., Hak, E., Schölvinck, E. H., & Niesters, H. G. M. (**2013**). The significance of rhinovirus detection in hospitalized children: clinical, epidemiological and virological features. Clinical Microbiology and Infection, 19(10), E435-E442.

Rammensee HG, Friede T, Stevanoviic S (**1995**) MHC ligands and peptide motifs: first listing. Immunogenetics 41: 178–228.

Randi AM, Hogg N (**1994**) I domain of beta 2 integrin lymphocyte function-associated antigen-1 contains a binding site for ligand intercellular adhesion molecule-1. J Biol Chem 269: 12395–12398.

Rappuoli, R. (**2000**). Reverse vaccinology. *Current opinion in microbiology*, *3*(5), 445-450.

Reed SE. (**1975)**. An investigation of the possible transmission of rhinovirus colds through indirect contact. J. Hyg. (Lond.) 75: 249–258

Renegar, K.B., Small Jr., P.A., Boykins, L.G., Wright, P.F., (**2004)**. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. J. Immunol. 173, 1978–1986.

Renwick N, Schweiger B, Kapoor V, Liu Z, Villari J, et al. (**2007**) A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. J Infect Dis 196: 1754–1760.

Rohde, G. G. U. (**2011**). Rhinovirus vaccination: the case in favour. *European Respiratory Journal*, *37*(1), 3-4.

Roivainen, M., Piirainen, L., Närvänen, A., Rysä, T. & Hovi, T. (**1993**)**.** An immunodominant N-terminal region of VP1 protein of poliovirion that is buried in crystal structure can be exposed in solution. *Virology* 195, 762–765.

Rossmann, M. G., E. Arnold, J. W. Erickson, E. A. Frankenberger, J. P. Griffith, H. J. Hecht, J. E. Johnson, G. Kamer, M. Luo, A. G. Mosser, R. R. Rueckert, B. Sherry, and G. Vriend. (**1985)**. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature (London) 317:145–153.

Rossmann MG, Olson NH, Kolatkar PR, Oliveira MA, Cheng RH, Greve JM, McClelland A, Baker TS. (**1994).** Crystallographic and cryo EM analysis of virion-receptor interactions. Arch Virol Suppl;9:531–541.

Savolainen, C., Blomqvist, S., Mulders, M.N. and Hovi, T. (**2002**). Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J. Gen. Virol. 83: 333-340.

Seechurn, P., Knowles, N. J., & McCauley, J. W. (**1990**).The complete nucleotide sequence of a pathogenic swine vesicular disease virus. *Virus research*, *16*(3), 255-274.

Senior , K.(**2002**). FDA panel rejects common cold treatment The Lancet infectious diseases, 2, p. 264

Sherry, B., and R. Rueckert.(**1985)**. Evidence for at least two dominant neutralization antigens on human rhinovirus 14. J. Virol. 53:137-143

Simmonds P., McIntyre C., Savolainen-Kopra C., Tapparel C., Mackay I. M., Hovi T. **(2010).**Proposals for the classification of human rhinovirus species C into genotypically assigned types.J Gen Virol 91, 2409–2419.

Singh, Surinder Mohan, and Amulya Kumar Panda.(**2005**). "Solubilization and refolding of bacterial inclusion body proteins." *Journal of bioscience and bioengineering*99, no. 4: 303-310.

Singleton RJ, Bulkow LR, Miernyk K, DeByle C, Pruitt L, Hummel KB, Bruden D, Englund JA, Anderson LJ, Lucher L, Holman RC, Hennessy TW.**(2010)**. Viral respiratory infections in hospitalized and community control children in Alaska. J. Med. Virol. 82:1282–1290

Skern, T., Neubauer, C., Frasel, L., Gründler, P., Sommergruber, W., Zorn, M., ...& Blaas, D. (**1987**). A neutralizing epitope on human rhinovirus type 2 includes

amino acid residues between 153 and 164 of virus capsid protein VP2.*The Journal of general virology*, *68*, 315-323.

Skern, T., Sommergruber, W., Blaas, D., Gruendler, P., Fraundorfer, F., Pieler, C., ...& Kuechler, E. (**1985**). Human rhinovirus 2: complete nucleotide sequence and proteolytic processing signals in the capsid protein region.*Nucleic Acids Research*, *13*(6), 2111-2126.

Smith TJ. Antibody interactions with rhinovirus. Semler BL, Wimmer E, eds.In*(***2002).** *Molecular Biology of Picornaviruses*. Washington, ASM Press, ; pp.39–49

Strassburg, M. A., Greenland, S., Sorvillo, F. J., Lieb, L. E., & Habel, L. A. (**1986**). Influenza in the elderly: report of an outbreak and a review of vaccine effectiveness reports. *Vaccine*, *4*(1), 38-44.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (**2011**). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution*, *28*(10), 2731-2739.

Tan BH, Loo LH, Lim EA, Kheng Seah SL, Lin RT, Tee NW, Sugrue RJ (**2009)**: Human rhinovirus group C in hospitalized children, Singapore**.***Emerg Infect Dis* , 15**:**1318-1320.

Tapparel, C., Junier, T., Gerlach, D., Cordey, S., Van Belle, S., Perrin, L., ...& Kaiser, L. (**2007**). New complete genome sequences of human rhinoviruses shed light on their phylogeny and genomic features. *BMC genomics*, *8*(1), 224.

Thibaut H.J., A.M. De Palma, J. Neyts. (**2012**).Combating enterovirus replication: state-of-the-art on antiviral research Biochem Pharmacol, 83, pp. 185–192

Turner RB, Lee W-M. (**2009).** Rhinovirus. In: Richman DD, Whitley RJ, Hayden FG, editors. Clinical Virology. Washington, DC: ASM Press;. pp. 1063–82

Traub S, Nikonova A, Carruthers A, Dunmore R, Vousden KA, et al. (**2013**) An Anti-Human ICAM-1 Antibody Inhibits Rhinovirus-Induced Exacerbations of Lung Inflammation. PLoS Pathog 9(8): e1003520. doi:10.1371/journal.ppat.1003520

Trent, C. A., Zimbro, K. S., & Rutledge, C. M. (**2014**).Barriers in Asthma Care for Pediatric Patients in Primary Care. *Journal of Pediatric Health Care*.

Toneatto, D., Oster, P., W deBoer, A. C., Emerson, A., Santos, G. F., Ypma, E., ...& Dull, P. (**2011**). Early clinical experience with a candidate meningococcal B recombinant vaccine (rMenB) in healthy adults. *Human vaccines*, *7*(7), 781-791.

Tormo, J., Blaas, D., Parry, N. R., Rowlands, D., Stuart, D., & Fita, I. (**1994**). Crystal structure of a human rhinovirus neutralizing antibody complexed with a peptide derived from viral capsid protein VP2. *The EMBO journal*, *13*(10), 2247.

Tuthill, T. J., D. Bubeck, D. J. Rowlands, and J. M. Hogle. (**2006)**. Characterization of early steps in the poliovirus infection process: receptor-decorated liposomes induce conversion of the virus to membrane-anchored entry-intermediate particles. J. Virol. 80:172-180.

Verdaguer N, Fita I, Reithmayer M, et al. (**2004)** X-ray structure of a minor group human rhinovirus bound to a fragment of its cellular receptor protein. Nat Struct Mol Biol ; 11: 429–434.

Walker, L. M., Huber, M., Doores, K. J., Falkowska, E., Pejchal, R., Julien, J. P., ...& Protocol G. Principal Investigators. (**2011**). Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature*, *477*(7365), 466-470.

Weber, C. A., Mehta, P. J., Ardito, M., Moise, L., Martin, B., & De Groot, A. S. (**2009**). T cell epitope: friend or foe? Immunogenicity of biologics in context.*Advanced drug delivery reviews*, *61*(11), 965-976.

Wei, H., Fang, M., Wan, M., Wang, H., Zhang, P., Hu, X., ...& Wang, L. (**2014**). Influence of hydrophilic amino acids and GC-content on expression of recombinant proteins used in vaccines against foot-and-mouth disease virus in Escherichia coli. *Biotechnology letters*, *36*(4), 723-729.

Welch, M., Villalobos, A., Gustafsson, C., &Minhull, J. (**2011**). 3 Designing Genes for Successful Protein Expression. *Methods in enzymology*, *498*, 43.

Whiteman, S.C., A. Bianco, R.A. Knight, M.A. Spiteri.(**2003**). Human rhinovirus selectively modulates membranous and soluble forms of its intercellular adhesion molecule-1 (ICAM-1) receptor to promote epithelial cell infectivity J. Biol. Chem., 278, pp. 11954–11961

Winther B, Gwaltney JM Jr, Mygind N, Turner RB, Hendley JO. (**1986).** Sites of rhinovirus recovery after point inoculation of the upper airway. *JAMA* ;256:1763–1767.

WorldHealth Organization.(**2013**). Asthma fact sheet. Retrieved from http://www.who.int/mediacentre/factsheets/fs307/en/

Wright, P. F., Tounkara, K., Lelenta, M., & Jeggo, M. H. (**1997**). International reference standards: antibody standards for the indirect enzyme-linked immunosorbent assay. *Revue scientifique et technique (International Office of Epizootics)*, *16*(3), 824-832.

Xiang Z, Gonzalez R, Xie Z, Xiao Y, Chen L, Li Y, et al (**2008)**. Human rhinovirus group C infection in children with lower respiratory tract infection. Emerg Infect Dis. ;14:1665–7

Yang X, Yu X ., (**2009).** An introduction to epitope prediction methods and software.*Rev Med Virol*; 19: 77–96.

Zhi, N., Wan, Z., Liu, X., Wong, S., Kim, D. J., Young, N. S., & Kajigaya, S. (**2010**). Codon optimization of human parvovirus B19 capsid genes greatly increases their expression in nonpermissive cells. *Journal of virology*, *84*(24), 13059-13062.

Zuckerman, A. J. (**2009**). *Principles and practice of clinical virology*. John Wiley & Sons