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

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By

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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.
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 subgroups, 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
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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 mengalakan 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.

Jujuukan 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 antibody 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 dilaikkan 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 mendapat 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 didapat 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 v
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 dibiarakan dan sangat mudah berubah.
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I certify that a Thesis Examination Committee has met on 12 August 2015 to conduct the final examination of Alshrai, 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.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>viii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxii</td>
</tr>
</tbody>
</table>

## CHAPTER

1 **INTRODUCTION**

2 **LITERATURE REVIEW**

   2.1 Overview of Picornaviruses 4
   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**

   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
3.3.2 Cloning and expression of the full-length
codon-optimized VP1-4 recombinant proteins
3.3.3 Extraction of recombinant proteins
3.3.4 Protein purification
3.4 Protein assays
3.4.1 Proteins concentrations
3.4.2 Protein purity
3.4.3 Staining protein gels
3.4.4 Western Blotting
3.5 Synthetic peptides
3.6 Production of antibodies against recombinant
VP1, VP2, VP3 and VP4 proteins and synthetic peptides
3.6.1 Ethics statement
3.6.2 Immunization of animals
3.7 Cell Culture and RV propagation
3.7.1 Growth and maintenance of H1-HeLa Cells
3.7.2 Virus propagation
3.7.3 Total RNA extraction
3.7.4 Measurement of RNA concentration and purity
3.7.5 Reverse-Transcriptase Polymerase Chain Reaction
(RT-PCR)
3.7.6 PCR Amplification of the cDNA
3.7.7 Agarose gel electrophoresis of RT-PCR product
3.7.8 PCR and RT-PCR products purification
3.7.9 Sequencing Analysis
3.8 Virus titration and determination of tissue culture
infectious dose (TCID50)
3.9 Immunoassay experiments
3.9.1 Investigation of antigen-specific rabbit antibodies
using indirect enzyme-linked immunosorbent Assay
(ELISA)
3.9.2 Cross-reactivity of rabbits anti-synthetic-peptides
with the recombinant proteins and vice versa
by indirect ELISA
3.9.3 Reactivity and cross-reactivity of the rabbit anti-
proteins and rabbit anti-peptides antisera with
virus particles by indirect ELISA
3.10 Rhinovirus Neutralization Assay In vitro
4 RESULTS
4.1 Bioinformatics analysis
4.1.1 Sequence analysis of VP1 Amino Acids of all RV
prototype strains
4.1.1.1 Sequence characterization of VP1
4.1.1.2 Conserved motifs of Rhinovirus VP1 protein
4.1.1.3 Phylogenetic analysis of rhinovirus
prototypes strains based on VP1 amino
acid sequences
4.1.1.4 Consensus sequences of VP1 compared with HRV-74 VP1
4.1.1.5 Phylogenetic tree of VP1 amino acid sequences of RV-A

4.1.2 Sequence analysis of VP2 Amino Acids of all RV prototype strains
4.1.2.1 Sequence characterization of VP2
4.1.2.2 Conserved motifs of Rhinovirus VP2 protein
4.1.2.3 Consensus sequences compared with HRV-74 VP2
4.1.2.4 Phylogenetic tree of VP2 amino acid sequences of RV-A

4.1.3 Sequence analysis of VP3 amino acids of all RV prototype strains
4.1.3.1 Sequence characterization of VP3
4.1.3.2 Conserved Motifs of Rhinovirus VP3 Protein
4.1.3.3 Consensus sequences of VP3 compared with HRV-74 Protein
4.1.3.4 Phylogenetic tree of VP3 amino acid sequences of RV-A

4.1.4 Sequence analysis of VP4 amino acids of All RV prototype strains
4.1.4.1 Sequence characterization of VP4
4.1.4.2 Conserved motifs of Rhinovirus VP4 protein
4.1.4.3 Consensus sequences of VP4 compared with HRV-74 Protein
4.1.4.4 Phylogenetic tree of VP4 amino acid sequences of RV-A

4.1.5 Selection of serotypes for immunoreactivity studies

4.2 Gene design, synthesis and expression vector construction for production of HRV-74 capsid recombinant proteins (VP1,VP2,VP3 and VP4)
4.2.1 Codon optimization and genes synthesis
4.2.2 Analysis of cloning and transformation of the full-length codon-optimized VP11-4 recombinant genes
4.2.3 Analysis of the recombinant fusion proteins
4.2.4 Proteins purity and concentrations

4.3 Synthetic peptides

4.4 Propagation of RVs
4.4.1 Detection of RV by RT-PCR

4.5 Induction of Antigen-Specific immune response in animals
4.5.1 Immune responses to recombinant proteins
4.5.2 Immune responses to synthetic peptides

4.6 Cross-reactivity of induced antibodies with their corresponding antigens and with various variant RV strains

4.7 Neutralization and cross neutralizing assay of Rhinovirus in vitro

xiv
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Taxonomic structure of the family <em>Picornaviridae</em></td>
<td>5</td>
</tr>
<tr>
<td>3.1</td>
<td>Dataset of the Rhinovirus prototype strains collected from the public database records</td>
<td>25</td>
</tr>
<tr>
<td>3.2</td>
<td>Profile of full-length codon-optimized VP1-4 sequences encoding native HRV-74 capsid proteins</td>
<td>29</td>
</tr>
<tr>
<td>3.3</td>
<td>A list of the synthetic peptides used for the experiment</td>
<td>33</td>
</tr>
<tr>
<td>3.4</td>
<td>Batches of the experimental animals and the corresponding antigens</td>
<td>35</td>
</tr>
<tr>
<td>3.5</td>
<td>Immunization procedure, dose and blood collection times</td>
<td>36</td>
</tr>
<tr>
<td>3.6</td>
<td>Selected serotypes represent different branches of the RV phylogeny</td>
<td>38</td>
</tr>
<tr>
<td>3.7</td>
<td>Set of primers used to amplify the VP4/VP2 region</td>
<td>40</td>
</tr>
<tr>
<td>4.1</td>
<td>Conserved sequences identified within RV-VP1 capsid proteins</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>RV serotype pairs with VP1 DPLQRDFLGLGHQWLRI</td>
<td>49</td>
</tr>
<tr>
<td>4.3</td>
<td>RV-$\text{SVHURWSHSDLUVZLWK93DPLQRDFLGLGHQWLRI}$</td>
<td>56</td>
</tr>
<tr>
<td>4.4</td>
<td>RV-$\text{-%VHURWSHSDLUVZLWK93DPLQRDFLGLGHQWLRI}$</td>
<td>56</td>
</tr>
<tr>
<td>4.5</td>
<td>Conserved sequences identified within RV-VP2 capsid proteins</td>
<td>58</td>
</tr>
<tr>
<td>4.6</td>
<td>RV serotype pairs ZLWK93DPLQRDFLGLGHQWLRI</td>
<td>64</td>
</tr>
<tr>
<td>4.7</td>
<td>Conserved sequences identified within RV-VP3 capsid proteins</td>
<td>66</td>
</tr>
<tr>
<td>4.8</td>
<td>RV-A serotypes whose identical by 100% to HRV-74 based on VP4 aa</td>
<td>72</td>
</tr>
<tr>
<td>4.9</td>
<td>Conserved motifs identified within RV-VP4 capsid protein</td>
<td>73</td>
</tr>
<tr>
<td>4.10</td>
<td>Dataset of selected serotypes represent different branches of the RV phylogeny</td>
<td>77</td>
</tr>
<tr>
<td>4.11</td>
<td>Profile of full length codon-optimized VP1-4 sequences encoding native HRV-74 capsid proteins</td>
<td>78</td>
</tr>
<tr>
<td>4.12</td>
<td>Codon distribution of VP1, VP2, VP3 and VP4 sequences</td>
<td>79</td>
</tr>
<tr>
<td>4.13</td>
<td>Profile of the recombinants proteins based on purity and concentration</td>
<td>87</td>
</tr>
<tr>
<td>4.14</td>
<td>Dataset of the synthetic peptides used for the experiment</td>
<td>89</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

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

4.15 Alignment of consensus of the RV-B VP2 sequence and HRV-74 VP2 sequence.

4.16 Phylogenetic tree of RV-A serotypes based on VP2 amino acid sequence.

4.17 Similarity of HRV-74 VP3 (aa) with other serotypes within RV-A.

4.18 Entropy plot of the full-length VP3 amino acid sequence alignment of all RV-A serotypes.

4.19 Entropy plot of the full-length VP3 amino acid sequence alignment of all RV-B.

4.20 Similarity of VP3 (aa) consensus sequence with all serotypes within RV-A.

4.21 Alignment of consensus of the RV-A VP3 sequence and HRV-74 VP3 sequence.

4.22 Alignment of consensus of the RV-B VP3 sequence and HRV-74 VP3 sequence.

4.23 Phylogenetic tree of RV-A serotypes based on VP3 amino acid sequence.

4.24 Median identity of VP4 aa of each serotype within RV-A.

4.25 Similarity of HRV-74 VP4 (aa) with other serotypes within RV-A.

4.26 Alignment of consensus of the RV-A VP4 and HRV-74 VP4 sequence.

4.27 Entropy plot of the full-length VP4 amino acid sequence alignment of all RV-A serotypes.

4.28 Entropy plot of the full-length VP4 amino acid sequence alignment of all RV-B serotypes.

4.29 Alignment of consensus of the RV-B VP4 and HRV-74 VP4 sequence.

4.30 Phylogenetic tree of RV-A serotypes based on VP4 sequence.

4.31 *E. coli* BL21 colonies after transformation of constructed cloning vectors.

xviii
4.32 Representative results show analysis of single colonies by PCR.

4.33 Representative result of purified constructed expression vectors.

4.34 Representative results show SDS-PAGE profile of E. coli BL21 proteins transformed with pET21a-VP1.

4.35 Representative results show SDS-PAGE profile of E. coli BL21 proteins transformed with pET21a-VP2.

4.36 Representative results show SDS-PAGE profile of E. coli BL21 proteins transformed with pET21a-VP3.

4.37 Representative results show SDS-PAGE profile of E. coli BL21 proteins transformed with pET21a-VP4.

4.38 Representative results for propagation of ATCC RVs using H1HeLa cell (magnification of 200x).

4.39 Representative results for amplification of VP4/VP2 fragments of RVs strains using RT-PCR.

4.40 Specificity of produced antibodies against recombinant VP1 and VP4 proteins.

4.41 Anti-VP1 protein IgM antibodies reacted specifically with recombinant VP1 protein.

4.42 Anti-VP1 protein IgG antibodies reacted specifically with recombinant VP1 protein.

4.43 Anti-VP4 protein IgM reacted specifically with recombinant VP4 protein.

4.44 Anti-VP4 protein IgG antibodies reacted specifically with recombinant VP4 protein.

4.45 Anti-VP2 IgM antibodies raised against recombinant VP2 or combination of VP1/VP2 proteins reacted specifically with recombinant VP2 protein.

4.46 Anti-VP1 IgM antibodies raised against combination of recombinant VP1/2 and VP1/3 reacted specifically with recombinant VP1 protein.

4.47 Anti-VP3 IgM antibodies raised against recombinant VP3 or combination of VP1/3 reacted specifically with recombinant VP3 protein.

4.48 Anti-VP1/2 and anti-VP1/3 IgM antibodies raised against combination of recombinant VP1/VP2 and VP1/VP3.
4.49 Anti-VP1/VP4 IgM antibodies raised against a combination of recombinant VP1 and VP4 proteins.

4.50 Anti-VP1/VP4 IgM antibodies raised against a combination of recombinant VP1 and VP4 proteins.

4.51 Anti-VP2 & anti-VP1/2 IgM antibodies raised against VP2 protein and combination of recombinant VP1/2 proteins.

4.52 Anti-VP3 & anti-VP1/3 IgM antibodies raised against VP3 protein and combination of recombinant VP1/3 proteins.

4.53 IgM antibodies raised against combination of (VP1-4) recombinant proteins.

4.54 IgG antibodies raised against combination of (VP1-4) recombinant proteins.

4.55 Specificity of produced antibodies against VP4 synthetic peptides.

4.56 Antisera IgM&IgG obtained from different groups immunized with synthetic peptides corresponding to VP1.

4.57 Antisera IgM&IgG obtained from different groups immunized with synthetic peptides corresponding to VP4.

4.58 Antisera IgM&IgG obtained from two groups immunized with synthetic peptides corresponding to VP2.

4.59 Representative result of antisera IgM obtained from two groups immunized with synthetic peptides corresponding to VP1.

4.60 Representative result of antisera IgG obtained from two groups immunized with synthetic peptides corresponding to VP4.

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.

4.62 Representative result of antisera IgG obtained from groups immunized with synthetic peptides or their corresponding proteins.

4.63 Inhibition of HRV-74 viral infectivity.

4.64 Inhibition of HRV-15 viral infectivity.

4.65 Inhibition of HRV-16 viral infectivity.

4.66 Inhibition of HRV-44 viral infectivity.
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  
dH₂O  Distilled water  
dNTPs  Deoxynucleotide triphosphate  
dsDNA  Double strand DNA  
ELISA  Enzyme-linked immunosorbent 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
H₂SO₄
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

xxiii
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM</td>
<td>Minimum essential medium</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
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<tr>
<td>MOI</td>
<td>Multiplicity of infection</td>
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<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<tr>
<td>MW</td>
<td>Moleculer weight</td>
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<tr>
<td>N-terminus</td>
<td>Amino terminus</td>
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<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>NCR</td>
<td>Non-coding region</td>
</tr>
<tr>
<td>NdeI</td>
<td>Restriction enzyme sites</td>
</tr>
<tr>
<td>Ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NIm</td>
<td>Neutralizing immunogenic site</td>
</tr>
<tr>
<td>NJ</td>
<td>Neighbour-joining</td>
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<tr>
<td>Nm</td>
<td>Nanometer</td>
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<tr>
<td>OD</td>
<td>Optical density</td>
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<tr>
<td>ORF</td>
<td>Open reading frame</td>
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<tr>
<td>qPCR</td>
<td>Quantitative real-time polymerase chain reaction</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PBST</td>
<td>Phosphate-buffered saline and Tween 20</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene difluoride</td>
</tr>
<tr>
<td>RDRP</td>
<td>RNA-dependent RNA polymerase</td>
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<tr>
<td>RG</td>
<td>Rabbit group</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>Rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>RV</td>
<td>Rhinovirus</td>
</tr>
<tr>
<td>RV-A</td>
<td>Rhinovirus group A</td>
</tr>
<tr>
<td>RV-B</td>
<td>Rhinovirus group B</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RV-C</td>
<td>Rhinovirus group C</td>
</tr>
<tr>
<td>RVs</td>
<td>Rhinoviruses</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SVDV</td>
<td>Swine vesicular disease virus</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-Borate-EDTA</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris Buffered saline</td>
</tr>
<tr>
<td>TCID</td>
<td>Tissue culture infectious dose</td>
</tr>
<tr>
<td>TMB</td>
<td>Tris-Borate-EDTA</td>
</tr>
<tr>
<td>URT</td>
<td>Upper respiratory tracts</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
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<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>VRIs</td>
<td>Viral respiratory infections</td>
</tr>
<tr>
<td>VP</td>
<td>Virus protein</td>
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<tr>
<td>VPG</td>
<td>Viral priming protein</td>
</tr>
<tr>
<td>XhoI</td>
<td>Restriction enzyme sites</td>
</tr>
</tbody>
</table>
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 are also 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 of small 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, which only 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 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 β-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 settling 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 by identifying five proteins that are conserved across the strains (Please refer to Giuliani et al., 2006; Toneatto et al.,
Thus, the current study was started with the bioinformatics approach to analyse all the expected immunogenic capsid proteins and identify the highly conserved ones and the ideal strain. The most likely antigenic and highly conserved sites within those capsid proteins were considered as vaccine candidates. Since RV is a diverse pathogen with more than 160 confirmed strains, the study initially focused on identifying the most suitable vaccine candidate for RV-A species as it is the most predominant group, without neglecting RV-B.

In the second stage and as an experimental validation, the four recombinant capsid proteins VP1-4 of the ideal strain were constructed and produced in vitro upon codon-optimized for E. coli so as to investigate their potential usefulness for vaccination against the common cold. Synthesising such codon-optimized genes and expressing them in a foreign host can shorten the vaccine production process under controlled conditions and at a reasonable cost. These recombinant proteins were then used to investigate whether they could induce cross-protective antibody against RVs. On the other hand, the highly conserved peptides corresponding to one or another of the four capsid proteins were also synthesised and experimentally tested for their potential ability to elicit antibodies cross neutralising the viral infectivity.

Objectives of the Study

General Objective
In general, this study was carried out to develop a pan-serotypic vaccine that is able to induce the production of cross-reactive antibodies covering all or most of the RVs serotype infections.

Specific Objectives
The current study was done with the following objectives:

1. To characterise the VP1, VP2, VP3 and VP4 proteins and their recombinants of different RV serotypes using the Bioinformatics tools.
2. To identify the highly conserved regions within the RVs capsid proteins.
3. To construct the recombinant VP1, VP2, VP3 and VP4 proteins from RV strain that is capable of inducing a serotype-specific immune response.
4. To construct immunogenic synthetic peptides corresponding to capsid proteins capable of inducing a specific immune response to RV infections.
5. To investigate whether the recombinant VP1, VP2, VP3 and VP4 proteins and the synthetic peptides are able to elicit antibodies cross-neutralising the viral infectivity in vitro.
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143


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