Objective 3. Develop new and improved diagnostic tools, vaccines, and novel management approaches

Development of novel nanoparticle-base vaccines for infectious bronchitis

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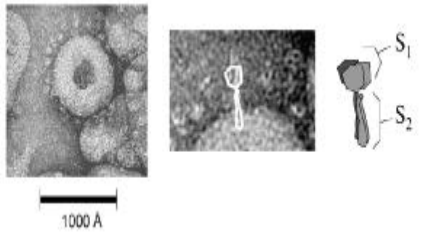
Infectious Bronchitis



IBV genome encodes four major structural proteins, known as spike (S) protein, nucleocapsid (N) protein, membrane (M) protein and envelope (E) protein.

The S protein is post-translationally cleaved into the outer S1 and the membrane bound S2 proteins. S1 protein comprises major antigenic determinants that induce neutralizing antibodies which make it a major target of vaccine design and immune therapy. S2 protein is conserved and comprises epitopes inducing cross-reactive Abs and cell –mediated immune responses.

N protein is largely conserved and contains epitopes which induce cytotoxic T lymphocyte (CTL) responses and help in protection as well as activating B cell.

To generate a potent vaccine the best conserved and protective B and T cell epitopes should be combined into a highly immunogenic epitope delivery and presenting system. The S protein of IBV contains two coiled-coil sequences.

Thus, these nanoparticles will present this epitope in a conformation-specific manner to the immune system, hence inducing conformation-specific antibodies with the potential to neutralize the virus in a viral infectivity assay

Virus Replication

IBV replicate in the cytoplasm, six messenger RNAs being produced by a discontinues transcription mechanism that can generate recombinants

Virion formation occurs by a budding process at the membranes of endoplasmic reticulum, not at the cell surface

Mechanism virion release from the cell is unknown

Design of IBV Nanoparticle based vaccine prototypes

Sequence analysis of the IBV surface protein

in order to identify the best B cell epitope to be displayed on the self-assembling protein nanoparticles (SAPN)

AF006624 (Keeler,C.L. Jr., Reed,K.L., Nix,W.A. and Gelb,J. Jr. *Serotype Identification of Avian Infectious Bronchitis Virus (IBV)*),

L10384(Jia,W., Karaca,K., Parrish,C.R. and Naqi,S.A. A novel variant of avian infectious bronchitis virus resulting from recombination among three different strains. Arch. Virol. 140 (2), 259-271 (1995))

L18990 Wang,L., Junker,D., Hock,L., Ebiary,E. and Collisson,E.W. *Evolutionary implications of genetic variations in the S1 gene of infectious bronchitis virus.* Virus Res. 34 (3), 327-338 (1994) and

M21883 (Niesters,H.G., Lenstra,J.A., Spaan,W.J., Zijderveld,A.J., Bleumink-Pluym,N.M., Hong,F., van Scharrenburg,G.J., Horzinek,M.C. and van der Zeijst,B.A. *The peplomer protein sequence of the M41 strain of coronavirus IBV and its comparison with Beaudette strains.* Virus Res. 5 (2-3), 253-263 (1986).

These sequences have also been compared to protein X-ray structures of the surface proteins of the viruses SARS and MERS

Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Li, F., Li, W., Farzan, M., Harrison, S.C. (2005) Science **309:** 1864-1868)

4NJL, 4KQZ, 4L3N, 4L72 for MERS (Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Lu, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Bao, J., Zhang, B., Shi, Y., Yan, J., Gao, G.F. (2013) Nature 500: 227-231

Research plan

The S protein of IBV contains two coiled-coil sequences as the S protein of SARS.

The second sequence (residues 1056-1083: **ILDIDSEIDRIQGVIQGLNDSLIDLEKL**) corresponds to the HRC sequence in SARS' S protein (residues 1158–1185: **VVNIQKEIDRLNEVAKNL NESLIDLQEL**) and shares a high sequence homology.

We have engineered this coiled-coil sequence onto the trimeric coiled-coil of several versions of our current SAPNs.

Thus, these nanoparticles will present this epitope in a conformation-specific manner to the immune system, hence inducing conformation-specific antibodies with the potential to neutralize the virus in a viral infectivity assay.

Research plan

A sequence alignment of these proteins revealed the coiled-coil heptad repeat regions of the proteins and the relevant portions of the head domain of the glycoprotein

A sufficient sequences similarity between the different structures revealed that the optimal B cell determinant within IBV to be used in a design of an IBV prototype vaccine are the coiled-coil sequences of the stalk domain.

The scheme below shows the sequence alignment of the IBV strains M21883 and L10384 with the SARS protein.

The coiled coil region is indicated with the heptad repeat pattern (**a**bc**d**efg) above the sequences

In red the coiled-coil sequence of SARS is highlighted that had been used in previous nanoparticle vaccine candidates for SARS

Below the sequences the symbols for the sequence conservation between the three different strains is indicated with “*” marking completely conserved residues.

```
                                    a  d          a  d          a  d          a  d
IBV-M2188   KHELPDFDKFN- YTVPILDIDSEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWLA    1099
IBV-L10384  KHELPDFDKFN- YTVPILDIDSEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWLA    1105
SARS        TSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLG 1187
                .  *:..:. ..          .:::*:.****::     * :.    **:*****::*. :     **********.
```

```
IBV-M2188       KHELPDFDKFN-YTVP***ILDIDSEIDRIQGVIQGLNDSLIDLEKLSILK***TYIKWPWYVWLA 1099
IBV-L10384      KHELPDFDKFN-YTVPILDIDSEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWLA 1105
SARS            TSPDVDLGDISGIN**ASVVNIQKEIDRLNEVAKNLNES**LIDLQELGKYEQYIKWPWYVWLG 1187
                     .  *:..:. ..        .:.:*:.****::    * :.   **:*****::*. :    **********.
```

As can be seen from this alignment the coiled-coil heptad repeat pattern in IBV (italic-bold-blue) is longer than the sequence that had been used in the design of the SARS nanoparticle vaccine prototype.

Also, after the heptad repeat pattern, a significant portion of the proteins are highly conserved between the different strains/viruses.

These highly conserved regions are likely very important for the function of the glycoprotein of the virus and hence it was decided to keep those sequences at least in some designs of a prototype IBV nanoparticle vaccine.

Below are the two protein sequences that were ordered as a gene to be sub-cloned into our expression plasmid for the bio-production of the nanoparticle proteins

IBV long-short

```
                                                        d   a   d   a   d   a   d   a d
MGHHHHHHHHHHGSWEEWNARWDEWENDWNDWREDWQAWRDDWARWRATWRRGRLLSRLERLERRLEELRRLLQLIRHENRMVL
  a   d   a   d   a   d   a   d   a
QFVRALSMQILDIDSEIDRIQGVIQGLNDSLIDLEKLSI
```

IBV short-long

```
                                                      d   a   d   a   d   a   d   a
MGHHHHHHHHHHGSWEEWNARWDEWENDWNDWREDWQAWRDDWARWRATWRRGRLLSRLERLERRLEEIRHENRMVLQFILDID
  a   d   a   d   a   d   a   d
SEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWY
```

Black: His-tag; **Green**:pentameric coiled coil; **Blue**:trimeric coiled coil; **Magenta**:CD4 T cell epitope; **Red**: B cell epitope; **Underscore**:Restriction site for sub-cloning
Heptad: The repeat pattern is indicated above the sequences (a d a d …)

From these two protein sequence the combinations “short-short” and “long-long” can then be easily generated using the suitable restriction sites.

IBV long-long

MGHHHHHHHHHHGSWEEWNARWDEWENDWNDWREDWQAWRDDWARWRATWRRGRLLSRLERLERRLEELRRLLQLIRHENRMVLQFVRALSMQ*ILDIDSEIDRIQGVIQGLNDSLIDLEKLSILK*TYIKWPWY

IBV short-short

MGHHHHHHHHHHGSWEEWNARWDEWENDWNDWREDWQAWRDDWARWRATWRRGRLLSRLERLERRLEEIRHENRMVLQF*ILDID SEIDRIQGVIQGLNDSLIDLEKLSI*

Black: His-tag; **Green**: pentameric coiled coil; **Blue**: trimeric coiled coil; **Magenta**: CD4 T cell epitope; **Red**: B cell epitope; **Underscore**: Restriction site for sub-cloning
Heptad: The repeat pattern is indicated above the sequences (a d a d …)

IBV SAPN constructs

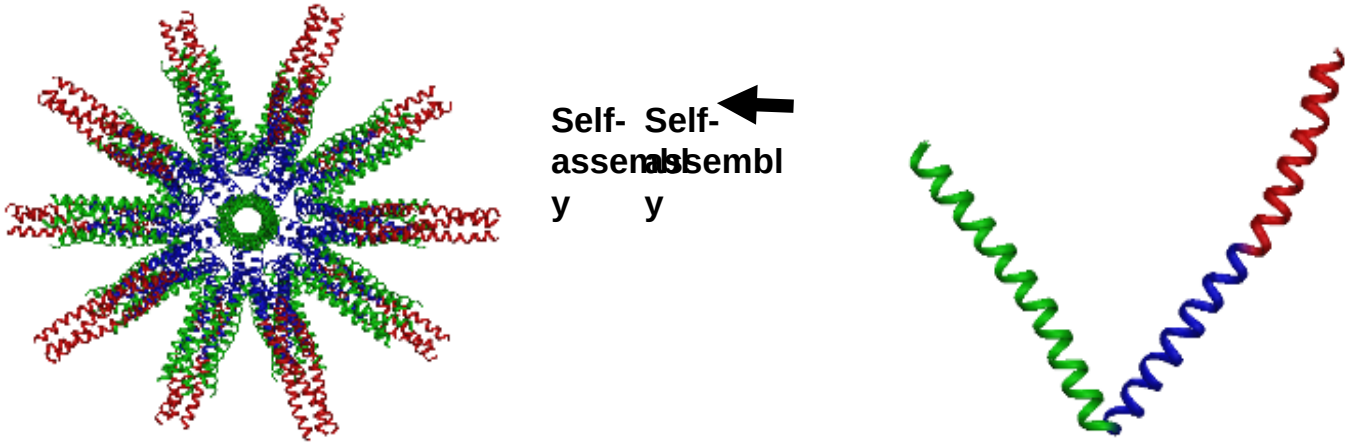


Figure 1. (**left**) 3D monomeric building block composed of a pentameric coiled-coil domain (green) and trimeric *de novo* designed coiled-coil domain (blue) which is extended by the coiled-coil sequence of the S protein of IBV (red); (**right**) Computer model of the protein nanoparticle icosahedral symmetry (not drawn to size with monomer).

Bioproduction of SAPN constructs

SAPN constructs have been designed and the nucleotide sequence of each SAPN were synthesized.

The oligos were ligated into the plasmid using suitable restriction sites and transformed to the competent DH5α cells.

Transformation, expression, purification and refolding are being performed as described in previous our studies.

Structural and biophysical analyses

Biophysical analyses will be performed on all constructs as soon as they become available from recombinant protein expression to confirm proper nanoparticle formation and to assess the stability and solubility properties of the SAPN constructs.

Analyses of the biophysical parameters will allow us (i) to determine the best constructs in terms of their stability, refolding properties and structural homogeneity, and (ii) to refine the expression, purification and refolding protocols to optimize nanoparticle formation using dynamic light scattering techniques, electron microscopy, and analytical ultracentrifugation.

Structural and biophysical analyses

Finally, melting curves of the peptide constructs at different concentrations will be measured by *CD Spectroscopy* to assess the stability of the SAPN.

After biophysical analyses of the SAPN vaccine constructs are completed, the best ones will be selected for immunogenicity studies in chickens.

The criteria for the selection of the constructs will be their aggregation behavior (i.e. the most soluble constructs), their ability to form nicely shaped and sized nanoparticles as well as their ease of expression in *E. coli*.