# The antigenic and genetic variability of bovine respiratory syncytial virus with emphasis on the G protein

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**ABSTRACT**: Bovine respiratory syncytial virus (BRSV) and related human respiratory syncytial virus (HRSV) are major respiratory tract pathogens in calves and infants, respectively. Great attention is now paid to prevention of the disease caused by these agents. Glycoprotein G is the most variable viral protein and antigenic grouping of RSV isolates is based on distinct antigenic reactivity patterns determined with a set of G protein specific mAbs. Genetic variability of the G protein is used during epidemiology and epizootiology studies of HRSV and BRSV diseases, respectively. The constant genetic drift can be observed within G protein sequences. Both cell-mediated and antibody-mediated immune responses contribute to efficient protection against RSV infection. The neutralizing antibodies are induced by F and G proteins. The G protein fails to induce cytotoxic lymphocytes response and may causes aberrant Th2 response leading to enhancement of clinical symptoms in subsequently infected vaccines. The G as the most variable viral protein associated with immunopathologic effect is a critical factor in vaccine development.

**Keywords**: bovine respiratory syncytial virus; glycoprotein G; antigenic variability; genetic variability; immune response

**Abbreviations**: BRSV – bovine respiratory syncytial virus, HRSV – human respiratory syncytial virus, ORSV – ovine respiratory syncytial virus, mRNA – messenger RNA, mAb – monoclonal antibody, FI – formalin-inactivated

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## 1. INTRODUCTION

Bovine respiratory syncytial virus (BRSV) is an important pathogen implicated in the respiratory syndrome in cattle. BRSV is related to human respiratory syncytial virus (HRSV) with whom it shares some common clinical, pathological and epidemiological characteristics. The viruses cause infections accompanied with bronchiolitis and pneumonia, the course of which might be severe especially in children and calves. Both the viruses are ubiquitous and are characterized by reinfections with winter outbreaks. The protein composition of both the viruses seems to be identical (Lerch *et al.*,

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1989) and some structural proteins show antigenic cross reactions (Baker *et al*., 1992; Stine *et al*., 1997). Despite these similar characteristics, the viruses are classified into separate antigenic groups confirmed by means of monoclonal antibodies and sequence analyses (Lerch *et al*., 1990; Stine *et al*., 1997).

Great attention is paid to the study of human respiratory syncytial virus regarding its impact on human health, especially in children, the elderly and immuno-compromised patients. At present, attention is paid to the circulation of the virus in the population and to the development of an efficacious vaccine. The first experiments with formalin-inactivated and alum-precipitated vaccine lot 100 applied to children not only failed to protect against wild-type RSV disease but also induced an exaggerated clinical response to wild-type RSV infection in infants who were RSV naïve before vaccination (Kim *et al*., 1969). This unexpected response to vaccination showed the need for better understanding of the pathogenesis and immune response of an organism to the infection. Immunological studies were performed predominantly on mice, whose response to the infection is not always identical to that of a natural host. Thus BRSV infection in calves could be a suitable homologous model for the study of immune processes in natural hosts (Graham *et al*., 2002; Taylor *et al*., 1997). For vaccine development it is necessary to know the antigenic variability of the viruses circulating in the population and its effect on protection of individuals following infection with a heterologous viral strain. From the standpoint of vaccine development, the attachment glycoprotein G and the fusion protein F of the virus have a leading role. The G protein is the most variable of the viral proteins, and its immuno-pathologic role in infections is being considered (Graham *et al*., 2000).

This paper provides an extension of previously reviewed literature about BRSV (Ellis *et al*., 2001; Kovarcik *et al*., 1997a,b; Larsen, 2000). After a description of general properties of the glycoprotein G, this review covers their consequences on epizootiology and immune response to infection.

## 2. VIRAL STRUCTURE

BRSV is a member of the Pneumovirus genus within the subfamily Pneumovirinae of the family Paramyxoviridae that belongs to the order of Mononegavirales. The virus is composed of a nucleocapsid and a viral envelope, and is considerably pleomorphic (Ito *et al*., 1973). The genome consists of a negative single-stranded RNA of about 15 Kbp, which encodes 10 viral proteins (Lerch *et al*., 1989). Viral proteins, except NS1 and NS2, are structural proteins. Nucleocapsid consists, besides RNA, of nucleoprotein (N), phosphoprotein (P) and RNA polymerase (L). Two major membrane proteins, the fusion protein (F), the attachment glycoprotein (G) and further the small hydrophobic protein (SH) and M2 protein are associated with the viral envelope. The F and G proteins stimulate production of cellular and humoral immune responses, and play a role in protection. Glycoprotein G is the attachment protein, responsible for binding the virus to a permissive cell. Glycoprotein F is responsible for the penetration of the virus into the host cell and the spread of the virus in organism and the formation of characteristic syncytia.

## 3. VIRAL PROTEINS

### 3.1 Glycoprotein G

Glycoprotein G is a unique protein among the viral attachment proteins. Unlike of other Paramyxoviridae proteins it lacks both haemaglutination and neuraminidase activity, its amino acid composition is not homologous with any other paramyxovirus proteins, and extensive glycosylation gives the G protein features similar to those of the mucinous proteins of the respiratory tract. Glycoprotein G exists in two forms, as an anchored, type II transmembrane protein, and as a small soluble protein secreted to the medium (Hendricks *et al*., 1987). Both the protein forms are translated from one mRNA using two different AUG initiation codons near the 5` end of the ORF gene in glycoprotein G. (Roberts *et al*., 1994).

Glycoprotein G is regarded as the attachment virus protein due to the inhibition of virus absorption by specific antibodies against glycoprotein G (Levine *et al*., 1987). The study of its function during replication of the virus revealed that glycoprotein G is dispensable for infection although infection using the virus with deleted glycoprotein G was changed due to the reduced efficiency in binding of the virus to cells (Lerch *et al*., 1990; Techaarpornkul *et al*., 2001; Teng and Collins, 2002). In addition, glycoprotein G takes part in completion and release of the virus from a cell (Techaarpornkul *et al*., 2001).

### 3.1.1 G protein structure

Messenger RNA (mRNA) for BRSV glycoprotein G of 383 bp contains one major open reading frame (ORF) encoding a protein with 257 AA. The suggested molecular weight of this protein is 28 kDa. Mature glycoprotein G isolated from infected cell culture is glycosylated, with an electrophoretic mobility of 80–90 kDa. A high percentage of hydroxy amino acids, serine and threonine, in the derived AA sequence of protein suggests that BRSV glycoprotein G is largely glycosylated, predominantly by means of O-linked sugars (80%), just as in HRSV.

Based on the similarity of the "hydropathy profile" in bovine and human RSV we can assume that the general structure of both the proteins is identical (Doreleijers *et al*., 1996; Lerch *et al*., 1990). Glycoprotein G is composed of three parts (domains): cytoplasmatic (AA 1-37), transmembrane (AA 38-65) and extracellular (AA 66-257) (ectodomain) (Langedijk *et al*., 1996). The cytoplasmatic domain is placed inside the viral envelope, the transmembrane domain runs across the cytoplasmatic membrane of the envelope. Both these sections of the protein are considerably conserved (Stine *et al*., 1997). The extracellular part of glycoprotein G is responsible for binding the virus to the sensitive cells, and the greatest differences within all viral proteins are localized there (Lerch *et al*., 1990). The ectodomain of the virus consists of a hydrophobic, conserved central area, flanked by hydrophilic variable regions (Doreleijers *et al*., 1996; Langedijk *et al*., 1996). The variable regions of the protein are called mucin-like due to their high content of carbohydrate side-chains bounded to Ser, Thr (O-linked sugars), and Pro (N-linked sugars). The central conserved region (AA 158-189) exhibits a characteristic globular structure with two cystein bridges (Cys 173-Cys 186 external, Cys 176-cys 182 internal) forming the "cystein noose" structure. The formation of this region starts with one turn of α helix (Cys 173-Cys 176) which runs anti-parallel to 1.5 α helix turns formed by Leu 180-Leu 185. The two helixes are further linked by a reverse type I turn (Cys 176-Asn 179). The connected helixes form a flat disc with a hydrophobic pocket flanked with conserved residues (Cys 176, Cys182, Leu185, Cys 186, Val 171, Pro 172, Cys 173). This pocket represents a suitable space for binding small ligands and most likely reacts with the presumed cell RSV receptor (Langedijk *et al*., 1996). The central conserved region is also an important immunodominant protein region. Non-synonymous mutations of more or less key residues (Cys 176, Cys 182, Leu 180 etc.) in this region have a great impact on protein antigenic reactivity.

Although the specific cell receptor has not been identified yet, it was found that the respiratory syncytial viruses are bound through glycosaminoglycans in the cell surface of the target cells (Karger *et al*., 2001). The domain responsible for the attachment activity of glycoprotein G is not known either. Most of the protein ectodomain is formed by two mucin-like regions flanking the central domain. In all human RSV the central domain contains 13 conserved amino acids, and four cystein residues forming a "cystein noose" present in all respiratory viruses (HRSV, BRSV, ORSV). This domain was considered an evident candidate carrying the attachment activity of the protein, and the cystein noose structure was considered necessary for binding the virus to the cell (Akerlind-Stopner *et al*., 1990). Using the deletion mutants in which the segments of glycoprotein G comprise all the four cystein residues or the cystein residues together with the conservative 13-amino acid sequence, Teng and others (Teng and Collins *et al*., 2002) demonstrated that the central conservative "cystein noose" is not necessary for an effective virus infection both *in vitro* and *in vivo*. It was assumed that the four cystein residues (Cys 173, Cys 176, Cys 182, Cys 186) are present in all human and bovine respiratory viruses. The culture of human viruses in the presence of monoclonal antibodies against glycoprotein G resulted in the selection of mutants with one cystein replaced by arginine (Rueda *et al*., 1994; Martinez *et al*., 1997). These mutant viruses were stable in further non-selective passages and grew with unchanged intensity in a mixed viral culture with the original virus. In addition, Valarcher and others (Valarcher *et al*., 2000) isolated viruses from diseased animals that had all four cystein residues replaced by arginine. Those substitutions led to structural changes in the central conserved region with the disturbance of α helix Cys 173-Cys 176. The other key residues of the central region remained unchanged. The authors assume that those changes are due to a higher positive selection pressure caused by vaccination, and an expression of virus evolution. In those viruses, antigenic reactivity was not determined but it can be assumed that it will be markedly changed due to the modification of antigenic epitopes (Langedijk *et al*., 1997). Experiments with mutant viruses with deleted glycoprotein G show that this glycoprotein

is not necessary for virus replication *in vitro*, although its replication is considerably decreased in both cell cultures and the respiratory tract of mice. It also seems that glycoprotein G is not the only virus protein with attachment activity, but both the glycoproteins G and F play role in attachment of the virus to cells (Karger *et al*., 2001; Techaarpornkul *et al*., 2001; Teng and Collins *et al*., 2002).

### 3.1.2. Genetic variability

Messenger RNA for glycoprotein G BRSV is of 383 bp and contains one major open reading frame coding for protein with 257 AA. Some of the viruses have the stop codon shifted by 18 nucleotides and thus the polypeptide chain is longer by 6 amino acid residues (Mallipeddi and Samal, 1993; Furze *et al*., 1997).

Numerous sequence analyses of both viruses were carried out to confirm the classification of viral strains into antigenic groups. Great differences among the amino acid sequences in individual representatives of antigenic groups were found in human viruses. In bovine strains the differences in protein sequences are not so marked and correspond to the heterogeneity of sequences within one group of HRSV (Furze *et al*., 1997).

The found range of sequence differences is rather variable. Furze *et al*. (1997) recorded 10 and 15% differences between nucleotide and amino acid sequences of BRSV viruses. On the other hand, the differences found by Stine *et al*. (1997) were lower with isolate identities 94.1–99.9% and 89.9–99.6% in nucleotide and protein sequences, respectively. These discrepancies are due to the selection of BRSV strains used for comparative analysis. Furze *et al*. (1997) compared European and American strains isolated during a longer period (1973–1986) while Stine *et al*. (1997) studied only BRSV viruses isolated in the USA over a ten-year period. Although genetic variability of viral proteins was studied predominantly in viruses passaged on cell cultures, we can see them as decisive because during the adaptation and passage of the virus no great changes of nucleotide sequences of viral proteins occur (Larsen *et al*., 1998).

Studies using synthetic peptides revealed that some amino acid residues (amino acids 174–185) of the central conserved region are immunodominant and the one-point substitutions in this region (especially residues 180, 183 and 184) have a great influence on antibody reactivity of single strains (Furze *et al*., 1997; Langedijk *et al*., 1997). An important mutation is also Ala205 → Thr205 located in variable region dividing individual strains into the groups A and AB+B (Langedijk *et al*., 1997) (Figure 1).

Variability along the G protein is not distributed proportionally. Most of the changes are localized in two regions of the gene, representing the mucin-like regions, those changes have the character of non-synonymous one-point mutations. On the contrary, the central hydrophobic region is more stable with prevailing synonymous mutations (Valarcher *et al*., 2000). Single nucleotide sequences differ in one-point substitutions. The escape mutants of HRSV selected by antibodies specific for glycoprotein G included further marked genetic changes: the premature stop codon, frame shift mutation, and A and G hypermutations (Garcia-Barreno *et al*., 1990; Martinez and Melero, 2002).

Both mucin-like regions of the protein have been shown to be of high antigenic importance in both BRSV and HRSV (Garcia-Barreno *et al*., 1990; Langedijk *et al*., 1996; Stine *et al*., 1997). The non-synonymous changes in these regions provide an opportunity for the virus to potentially escape from previously established immunity.

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                    170                                        209
391-2 (A)           YVPCSTCEGN**L**AC**LS**LCHIETERAPSRAPTITLKK**T**PKP
220-69 (AB)         ----------**S**---Q-GL----------**A**---
127 (B)             H---------**P**--**SP**--Q--L-----S--------**A**---
WBH (untyped)       H---------**P**--**S**---Q-GP---S----------
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Figure 1. Alignment of residues 170 to 209 of strains* 391-2, 220-69, 127 and WBH. The antigenic group of particular strains are are given in brackets. Residues with great influence on antibody reactivity of single strains are in bold

*the sequences of strains were taken from GenBank, accession Nos.: AAA42810 (391-2), Q84183 (220-69), CAA69966 (127) and O10687 (WBH)

### 3.1.3. Antigenic variability

HRSV strains can be divided into two major antigenic groups designated A and B. The differences in reactivity with the given monoclonal antibodies (mAbs) and in nucleotide sequences are expressed at certain levels in some proteins: the attachment proteins G, F and SH, nucleoprotein N and protein P (Morgan *et al*., 1987; Orvell *et al*., 1987; Cristina *et al*., 1990). The greatest differences were observed within glycoprotein G. Antigenic groups are in correlation with different genotypes (Mufson *et al*., 1985; Cristina *et al*., 1990; Gottschalk *et al*., 1996). A greater number of the examined viruses showed that they are not homogeneous within the one group (Morgan *et al*., 1987; Orvell *et al*., 1987; Cristina *et al*., 1990). Greater variability was generally found in viruses of the antigenic group A (Venter *et al*., 2001). Antigenic epitopes of glycoprotein G can be divided by means of monoclonal antibodies into conserved present in all HRSV isolates, group-specific shared by viruses from the same antigenic group and strain-specific or variable epitopes present in a limited set of viruses from the same antigenic group. The strain specific epitopes are localized mainly in variable regions compared to the conserved and group specific epitopes which are localized in the central region of glycoprotein G ectodomain (Akerlind-Stopner *et al*., 1990; Martinez *et al*., 1997; Jones *et al*., 2002).

Although antibodies against F, P, N and M protein in HRSV and BRSV can cross-react (Orvell *et al*., 1987), antibodies against glycoprotein G react only with homologous bovine or human isolates and the derived amino acid sequences of HRSV and BRSV glycoprotein G show only 29–30% identity, which indicates that BRSV belongs to another group than HRSV within pneumoviruses (Lerch *et al*., 1989, 1990; Baker *et al*., 1992).

The first reference to antigenic variability of BRSV strains was published by Lerch *et al*. (1989) who found that glycoprotein G of the strain 391-2 was recognized by a polyclonal serum against a homologous virus but not by a polyclonal serum against 127 BRSV strain. Baker *et al*. (1992) found by means of mAb against structural proteins of HRSV viruses (groups A and B) antigenic differences among nine BRSV strains and also recorded structural differences between F and P proteins of BRS viruses.

The first complex study concerning the antigenic heterogeneity of BRSV was published in by Furze *et al*. (1994) who classified 20 BRSV isolates into two major antigenic groups A, B and AB using the reactivity of mAb panel against G protein of BRSV strain 391-2 (mAb 44-69) and strain 127 (mAb 70, 75–79, 86, 87) in ELISA and RIPA tests. Viruses of group A reacted with all mAb against the strain 391-2 but the isolates of group B reacted only with mAb against the strain 127. Unlike viruses of group A, the viruses of group B did not react uniformly, only strains 127 and 4642 reacted with all 127-specific mAb, the other viruses reacted only with mAb 70, 78 and 86 (Furze *et al*., 1997). Besides these two major reactivity patterns with mAb, two exceptions were recorded, namely in the viruses 220-69 which react with some mAb of group A (except mAb 62, 46, 47, 57, 53, 61 and 69) and at the same time with mAb 70 and WBH which does not react with any of the mAb, nor with polyclonal sera against the strains 391-2 and 127 (Table 1). The strain 220-69 was classified in an independent intermediate group AB. Viruses of group B were isolated latest of all, in the 1970s. Polyclonal antibodies of calves vaccinated with recombinant vaccinia virus expressing glycoprotein G of a certain antigenic group are group specific.

Besides the antigenic differences between the glycoproteins G, differences in mAb reactivity against N, P, M and F proteins and differences in molecular weight of F and P proteins were also recorded. Structural differences among F proteins of single viruses are in correlation with the classification of viruses into antigenic groups, which is not the case of P protein (Furze *et al*., 1994; Stine *et al*., 1997). The variability of BRSV strains was also determined using RNase mismatch cleavage method (Cristina *et al*., 1990).

Monoclonal antibodies against glycoprotein G are used to determine the antigenic variability of bovine respiratory viruses, and recently several studies have been published on this subject (Schrijver *et al*., 1996a; Prozzi *et al*., 1997; Elvander *et al*., 1998; Larsen *et al*., 1998). The shortcoming of those studies is the determination of the reaction pattern of glycoprotein G with different mAb sets. However, the important fact is that some viruses cannot be classified (based on the determined reaction pattern) in any of the previously determined groups. Those viruses react only with some mAb specific for A group, but with another reaction pattern than that of the representatives of the intermediate AB group. The knowledge of that fact together with the high homology of nucleotide and amino acid sequences of G protein (Mallipeddi and Samal, 1993; Schrijver *et al*., 1996a,b; Prozzi *et al*., 1997; Elvander *et al*.,

Table 1. Binding of mAbs to particular antigenic groups of BRSV. Data are obtained from the references Furze *et al.* (1994, 1997)

| mAb | Antigenic group | | | |
|---|---|---|---|---|
| | A | AB | B* | |
| | | | I | II |
| 44 | ■ | ■ | | |
| 45 | ■ | ■ | | |
| 46 | ■ | | | |
| 47 | ■ | | | |
| 48 | ■ | ■ | | |
| 49 | ■ | ■ | | |
| 50 | ■ | ■ | | |
| 51 | ■ | ■ | | |
| 52 | ■ | ■ | | |
| 53 | ■ | | | |
| 54 | ■ | ■ | | |
| 55 | ■ | ■ | | |
| 56 | ■ | ■ | | |
| 57 | ■ | | | |
| 58 | ■ | ■ | | |
| 59 | ■ | ■ | | |
| 60 | ■ | ■ | | |
| 61 | ■ | | | |
| 62 | ■ | | | |
| 63 | ■ | ■ | | |
| 64 | ■ | ■ | | |
| 65 | ■ | ■ | | |
| 66 | ■ | ■ | | |
| 67 | ■ | ■ | | |
| 68 | ■ | ■ | | |
| 69 | ■ | | | |
| 70 | | ■ | ■ | ■ |
| 75 | ■ | | ■ | |
| 76 | ■ | | ■ | |
| 77 | ■ | | ■ | |
| 78 | ■ | | ■ | ■ |
| 79 | ■ | | ■ | |
| 86 | ■ | | ■ | ■ |
| 87 | ■ | | ■ | |

*different reactivity patterns of B strains according to Furze *et al*. (1997) are indicated

I = reactivity patterns of strains 127 and 4642

II = reactivity patterns of strains C881, X 21/72 and 220-60

1998; Larsen *et al*., 1998) is the major cause of doubts concerning whether BRSV viruses can be divided into different antigenic groups. This hypothesis is also supported by the fact that no significant differences in virulence have been observed so far among the determined antigenic groups as in HRSV viruses. Similar results were previously detected in human respiratory viruses too, when it seemed that viruses of a given antigenic group are homogenous. Increased amounts of data on different strains of the virus made us believe that antigenic and structural differences exist within individual groups (Morgan *et al*., 1987; Orvell *et al*., 1987; Storch *et al*., 1991).

The above-mentioned impossibility to classify some BRSV viruses into antigenic groups and two reactivity patterns of B group viruses with G-specific monoclonal antibodies suggest that similar antigenic differences also exist in BRS viruses. In addition, polyclonal sera from hyperimmune calves recognize only the G protein of strains belonging to the homologous groups by immunoprecipitation and sera from calves vaccinated with rVV expressing G of the A group isolate recognize A strains and to a lesser extent the intermediate strain (Furze *et al*., 1997). It is also probable that the viruses which are at present classified in group A and AB actually belong to one serological group with just some variable epitopes of glycoprotein G. Most of the monoclonal antibodies that determine the classification of single BRSV viruses in an antigenic group recognize the epitopes localized mainly in the central conserved area of the protein (Langedijk *et al*., 1997). The only exception is mAb 70 which detaches AB viruses. This mAb binds to the mucin like region of the protein as strain specific antibodies in HRSV. Further studies are needed to understand antigenic variability of BRSV. These should focus predominantly on the detection of monoclonal antibodies which bind to epitopes of variable protein regions.

### 3.2. Other proteins

Although the greatest differences are found in glycoprotein G of the virus, structural and antigenic heterogeneity can be also found in some other structural proteins. Most important of those proteins is the fusion protein F. Together with glycoprotein G, the F protein is expressed on the viral envelope and it is the key antigen for formation of neutralizing antibodies. The F protein is synthesized as an inactive precursor $F_0$ which is digested with cellular proteases to two subunits, $F_1$ and $F_2$ that are linked by disulfide bonds. The F protein of RSV viruses is highly conserved, the total homogeneity of F proteins in BRSV strains being >95% (Pastey

and Samal, 1993). The differences among F proteins are confined to different sizes of $F_2$ subunits which is conditioned by a different level of glycosylation in this subunit (Pastey and Samal, 1993). The classification of BRSV viruses according to size differences in F protein is in correlation with their classification in antigenic groups based on glycoprotein G (Furze *et al*., 1994). Antigenic differences in F protein are not significant. Structural and small antigenic differences exist also among P proteins (Baker *et al*., 1992; Furze *et al*., 1994; Shadomy *et al*., 1997). P protein is together with N protein needed for replication of viral RNA. Although size differences associated with classification in antigenic groups were found in HRSV viruses, no such thing was observed in bovine viruses.

## 4. EPIZOOTIOLOGY OF THE DISEASE

One of the most important features of the respiratory syncytial viruses is the ability to infect the host even in the presence of virus-neutralizing antibodies and to induce reinfections throughout the whole life (Beem *et al*., 1967).

Great attention has been so far paid to the behavior of HRSV with regard to its impact on human health. Few types of HRSV epidemics have been described so far: the epidemics in which only viruses of one antigenic group have been detected, the epidemics in which the viruses of one group only predominate, and the epidemics in which viruses of both groups circulate together with different frequency (Anderson *et al*., 1991). In each of these types of epidemics, co-circulation of different genotypes can be observed as is documented in the studies of several authors (Anderson *et al*., 1991; Cane and Pringle *et al*., 1991; Peret *et al*., 1998; Kamasaki *et al*., 2001; Roca *et al*., 2001; Seki *et al*., 2001; Venter *et al*., 2001). Genetic studies of successive epidemics showed that the dominant genotype of one epidemic is in successive epidemics replaced by a new genotype (Choi and Lee, 2000). A constant genetic drift can be observed within one genetic lineage isolated in the same community (Kamasaki *et al*., 2001). This genetic drift is expressed by a high degree (more than 50%) of non-synonymous substitutions with regard to the overall number of nucleotide changes which is markedly higher than we could assume in random nucleotide mutations. The same genetic drift can be observed in haemaglutinin and neuraminidase in influenza A viral strains (Air *et al*., 1990) which is the manifestation of selection pressure of the host immune system on viral proteins. On the other hand, single antigenic lineages can be identified in different countries in the course of several years (Anderson *et al*., 1991).

The variability of glycoprotein G sequences is in HRSV expressed predominantly by nucleotide substitutions and by the use of the alternative stop codon leading to the synthesis of glycoprotein G of different length (Peret *et al*., 1998; Martinez *et al*., 1999; Kamasaki *et al*., 2001). To a lesser extent, the deletion of one amino acid in the protein sequence can be observed in some isolates or frame shift mutation (Sullender *et al*., 1991). Changes in amino acid sequences result in antigenic differences among viruses (Martinez *et al*., 1999). The differences in protein glycosylation also contribute to antigenic variability (Sullender *et al*., 1991), which can lead to masking or, on the contrary, to exhibition of antigenic epitope of the protein (Palomo *et al*., 1991). The viruses of group A are being isolated more frequently than those of group B, which is most probably due to the higher variability of A viruses. It also seems that viruses of group A induce more severe infections than strains of group B (McConnochie *et al*., 1990). Moreover, it was found that viruses isolated in geographically distant places over several years can be more related than viruses isolated in one place over a few days (Melero *et al*., 1997). Those findings support the idea that HRSV strains circulate worldwide. Which of those circulating strains will induce infection in a certain community will most probably depend on local factors, especially on the specificity of immunity to the viral strain causing preceding infection (Anderson *et al*., 1991).

The pattern of BRSV transmission among individual infections has not been determined yet, different causes of recurrent infections are being considered. Some of the findings support the suggestion that BRSV is maintained in the herd either by population persistence (continuous transmission of the virus among animals) or by persistence of the virus in individual animals (van der Poel *et al*., 1997). On the other hand, Larsen (2000) characterized BRSV isolates during recurrent outbreaks by the use of G protein sequence data, and their results showed that identical viruses were isolated in a herd during single outbreak and viruses from recurrent infections varied by up to 11%. These results suggest that recurrent outbreaks are likely to be caused by different BRSV types newly introduced into herd.

## 5. IMMUNE RESPONSE

Great attention is being paid to the prevention of infection regarding the effect of the respiratory syncytial viruses on health of humans and animals. The first attempts with formalin-inactivated (FI) vaccine led in humans to a more severe course of the disease following a subsequent natural infection (Kim *et al*., 1969). The underlying mechanism of enhanced disease in FI vaccinated infants after subsequent naturally infection is not fully understood. However, the animal model suggests that it was due to strong T-cell priming which predominantly produces Th2 cytokines interleukin-4 (IL-4), IL-5, and IL-13 and induce eosinophilic response (Openshaw *et al*., 2001; De Swart *et al*., 2002). Such an unexpected effect of vaccination led to a more detailed study of the immune response to the respiratory syncytial viruses. Maternal antibodies commonly present in calves do not provide complete protection against infection (Kimman *et al*., 1988). Although the infection also occurs in the presence of neutralizing antibodies, they are considered protective as high titres decrease the severity of the disease in both children and calves (Kimman and Westenbrink, 1990). Cell-mediated immunity plays an important role in the pathogenesis of the infection. In mice it seems that T cells support the pathogenesis of the disease, especially glycoprotein G primed CD4+, by shifting the immune response more towards type 2 with a great proportion of eosinophils (Brandenburg *et al*., 2001; Hacking and Hull, 2002; Varga and Braciale *et al*., 2002). In experimentally infected calves the concentration of Th2 specific interleukin IL-4, 5 and IgE antibodies strongly correlated with increase in severity of clinical disease (Gershwin *et al*., 2000).

The immune response to HRSV infection has predominantly been studied in mice. However, the role of individual components of the immune system is not in precise agreement with the data obtained in the course of infection in natural hosts, especially since the mice with a depleted population of T cells showed prolonged shedding of the virus and less severe pulmonary lesions compared to immunocompromised humans and calves which show more severe lower respiratory tract disease (Englund *et al*., 1988; Graham *et al*., 1991; Taylor *et al*., 1995, 1997). The best model for elucidation of the pathogenesis of HRSV in humans seems to be BRSV infection in natural hosts – calves. A detailed study of specific immune response in BRSV infected calves revealed that predominantly MHC I class-restricted CD8+ cytotoxic lymphocytes play a dominant role in clearing the virus from lungs and nasopharynx. On the other hand, antibodies attenuated the severity of pathological changes in lungs (Taylor *et al*., 1995; Thomas *et al*., 1998; West *et al*., 1999). Following viral infection, the specific memory of T cells remains (Sandbulte and Roth, 2002); cytotoxic activity of T cells is independent of the antigenic variability of the virus (Gaddum *et al*., 1996). Different RS virus proteins prime for different virus-specific T cell responses and interleukin production and thus have an effect on the course of infection (Connors *et al*., 1992; Alwan *et al*., 1993; Taylor *et al*., 1997). Human cytotoxic T cell lymphocytes recognize the N, SH, F, M, M2 and NS2 proteins but not the G protein (Alwan *et al*., 1993; Hacking and Hull, 2002). The F and M2 proteins prime for Th1 type interleukins whereas G protein and to lesser extend N and P for Th2 type interleukins (Alwan *et al*., 1993). Infection with a live virus preferentially primes cells of Th1 type. So far the production of antibodies against F, G and N proteins has been confirmed in BRSV as well as lymphocyte proliferative responses in F, G, N and M2 proteins (Taylor *et al*., 1997). The G protein fails to induce cytotoxic lymphocytes response in both BRSV and HRSV (Taylor *et al*., 1998).

The neutralizing antibodies are induced by F and G proteins as were evaluated in calves vaccinated with rVV encoding these proteins. The high titre of neutralizing antibodies is induced by F protein, but only a low level of complement-dependent neutralizing antibodies are induced by G protein (Taylor *et al*., 1997; Thomas *et al*., 1998). Partially neutralizing antibodies are raised against the epitopes of glycoprotein G. These antibodies provide predominantly strain-specific immunity, although recombinant vaccines which express only G protein BRSV are able to induce complete protective immunity of the lower and to a lesser extent upper respiratory tract (Schrijver *et al*., 1996b; Taylor *et al*., 1998).

The above mentioned facts show that both cell-mediated and antibody- mediated immune response contribute to the efficient protection of animals. During the primary infection the immune response depends predominantly on cytotoxic activity of T lymphocytes which has only a short-time effect; long-time resistance is predominantly provided by antibodies (Gaddum *et al*., 1996). Schrijver *et al*. (1996b) found that G peptide specific antibodies decline more rapidly and to lower levels than

the F protein-specific antibodies, although both antibody titers peaked at the same time. Moreover, it is possible that low levels of G antibody render cattle susceptible to reinfection (Schrijver *et al*., 1996b). Therefore it seems that the high level of G-specific antibodies is very important for long-lasting immunity and it should be considered together with the high variability of G protein for vaccine design.

An efficient vaccine for RS viruses should induce a long-lasting humoral and Th1-specific cell-mediated immunity response to both the attachment proteins F and G. With regard to the immune pressure of a host organism on viral proteins which leads to marked antigenic changes predominantly in glycoprotein G (Valarcher *et al*., 2000), it is of great importance to use the latest viral strains isolated to ensure the high efficiency of the vaccine, although the current knowledge on BRSV indicates good protection of animals even after reinfection with a heterologous strains (Schrijver *et al*., 1998). On the other hand, the BRSV attachment glycoprotein G seems to be dispensable in vaccinating calves against BRSV (Schmidt *et al*., 2002).

## 6. CONCLUSIONS

BRSV and the related HRSV viruses are important pathogens causing respiratory infections. At present, great attention is paid to the development of an efficient vaccine against HRSV and to the study of pathogenesis of this infection. From the standpoint of vaccine development, the attachment glycoprotein G and the fusion protein F of the virus have a leading role. The attachment glycoprotein G is being most intensively studied due to its ability to evoke immunopathological reactions and its greatest variability among viral proteins.

HRSV and BRSV viruses are both classified into antigenic groups based on the variability of glycoprotein G. Using the panel of monoclonal antibodies raised against glycoprotein G, BRSV viruses can be divided into the groups A, B and AB. The extracellular domain of the protein has a characteristic structure with the conserved central part flanked with two variable mucin-like regions. The nonsynonymous mutations along the G protein gene of RSV are localized predominantly in mucin-like regions. The central hydrophobic region of the G protein has been shown to accept predominantly synonymous mutations, the nonsynonymous mutations in this region have a great impact on protein antigenic reactivity. The nonsynonymous mutations in mucine-like regions lead to antigenic changes presented among strains from the same antigenic group. On the other hand, nonsynonymous mutations in the central conserved region have a great influence on antibody reactivity. Division of viral strains into antigenic groups are mainly based on different reactivity of epitopes localized in this region. The nonsynonymous mutations provide an opportunity for the virus to potentially escape from previously established immunity.

In view of the fact that some BRSV isolates cannot be classified into the above antigenic groups, and because of the high homology among nucleotide and protein sequences of the virus, it is most likely that the current circulating viruses belong to one serological group.

Some of the characteristics of RSV infection is that reinfection occurs in human and bovine adults. The mode of trasmission during the cause of natural BRSV infection has not been explained yet. Studies made during epidemics of HRSV revealed that the dominant genotype of glycoprotein G is replaced in another outbreak of the infection by a new distinct genotype and genetic drift can be observed within one community.

Both humoral and cell-mediated immunity are involved in the immune response. A detailed study of specific immune response in BRSV infected calves revealed that predominantly MHC I class-restricted CD8+ cytotoxic lymphocytes play a dominant role in clearing the virus from lungs and nasopharynx. On the other hand, antibodies attenuated the severity of pathological changes in lungs. Enhancement of clinical signs of the infection in children following vaccination with inactivated virus suggested that immunopathological mechanism can occur after subsequent infection. Glycoprotein G is responsible for Th2 type of the immune response as it induces the formation of interleukins engaged in this type response. Broadly neutralizing antibodies are produced against F protein, antibodies against glycoprotein G induce predominantly strain specific immunity, but their titres have an influence on susceptibility to reinfection.

An efficient vaccine against the respiratory syncytial viruses should prime for both humoral and cell-mediated immunity. Protein G may cause aberrant Th2 response leading to enhancement of clinical symptoms in subsequently infected vaccines (Graham *et al*., 2000). On the other hand, it seems that protein G specific antibody protect from

reinfection (Schrijver *et al*., 1996). Both the protective and enhancing immune response to protein G should be considered in the process of developing RSV vaccines.

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## 8. REFERENCES

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