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# MHC Class I Ligands and Epitopes in HRSV Infection

Daniel López
*Centro Nacional de Microbiología, Instituto de Salud "Carlos III"*
*Spain*

## 1. Introduction

Human respiratory syncytial virus (HRSV) (Collins et al., 2007), a *Pneumovirus* of the *Paramyxoviridae* family is an enveloped virus containing a negative-sense, single-stranded RNA genome of 15.2 kilobases that is transcribed into 10mRNAs encoding 11 proteins because two overlapping open reading frames of M2 mRNA encode the M2-1 and M2-2 proteins. N is the nucleocapsid protein; L, the catalytic subunit of the RNA-dependent RNA polymerase, M2-1 transcription factor and the P phosphoprotein are components of the polymerase complex. M2-2 is a regulatory factor in RNA synthesis whereas M is the matrix protein. The non structural proteins NS1 and NS2 are antagonists of host type I interferons (IFN). Lastly F, G and SH are three transmembrane proteins.

HRSV is the single most important cause of serious lower respiratory tract illnesses such as bronchiolitis and pneumonia in infants and young children (Hall, 2001; Shay et al., 2001; Thompson et al., 2003). In these acute lower respiratory infections due to HRSV, the case fatality ratio in children younger than 1 year of industrialised or developing countries is the 0.7% and 2.1% respectively (Nair et al., 2010). Worldwide mortality from HRSV infection has been estimated in about 66,000 to 199,000 deaths annually in young children (Nair et al., 2010).

In addition, infections of this virus occur in people of all ages, but although usually mild infections are reported in healthy adults, HRSV poses a serious health risk in immunocompromised individuals (Ison & Hayden, 2002; Wendt & Hertz, 1995) and in the elderly (Falsey et al., 2005; Han et al., 1999). In the United States, HRSV is estimated to cause approximately 13,000 annual deaths among adults who are elderly or have underlying immunosuppressive and/or cardiopulmonary conditions (Falsey et al., 2005).

HRSV exists as a single serotype, but has two antigenic subgroups, A and B (Anderson et al., 1985; Mufson et al., 1985). The attachment G protein, a type II transmembrane glycoprotein with little homology to any other known viral protein is the major source of antigenic differences between the two HRSV subgroups. Between these subgroups A and B, the G protein varies by ~50% in amino acid sequence (Johnson et al., 1987). The nature of these differences indicates that the two subgroups represent two lines of divergent evolution, rather than variants that differ only at a few major antigenic sites.

Although the ciliated cells of the respiratory epithelium are the primary site of HRSV replication *in vivo* (reviewed in (Collins et al., 2007)), this virus can infect both human and

murine immune system cells, mainly professional antigen-presenting cells (APCs). HRSV infection induces maturation in human and murine monocytes and macrophages (Becker et al., 1991a; Franke-Ullmann et al., 1995; Midulla et al., 1989; Panuska et al., 1990) and human plasmacytoid but not myeloid dendritic cells (Hornung et al., 2004). Upregulation of typical activation markers such as CD86 and MHC class II upon HRSV infection of mouse spleen B cells was also previously reported (Rico et al., 2009; Rico et al., 2010).

## 2. Innate and humoral immunity

The HRSV infection, as any other pathogenic illness, is controlled by the concerted activity of different layers from host immune system. Cells of innate immune response as neutrophils infiltrate deeply the airways of ventilated HRSV-infected infants as shown their high presence in broncho-alveolar lavage samples of these young patients (Everard et al., 1994). Also, abundant alveolar macrophages were found in the lower respiratory tract in HRSV infection (Becker et al., 1991b). In addition, the depletion of macrophages enhances virus titers in the HRSV-infected lung by significant inhibition of early inflammatory cytokines release (Pribul et al., 2008).

Although the humoral response plays no role in the course of a primary infection, the protection from subsequent HRSV infections is mediated through antibodies (Abs). In mice model, the depletion of B lymphocytes does not alter the clearance of virus in the primary infection but the rate of viral clearance after secondary infection was significantly decreased (Graham et al., 1991a). Decreased protection from secondary infection correlated with low titers of HRSV-specific Abs in the serum of exposed individuals (Mills et al., 1971). Also, newborn infants with high titers of maternal acquired Abs are less likely to severe bronchiolitis (Holberg et al., 1991). In high-risk infants the passive immunization with HRSV-specific Abs reduces hospitalization from HRSV infection (The IMpact-RSV Study Group, 1998).

Vaccination studies using individual HRSV proteins have shown presence of serum Abs induced by F, G, M2 and P proteins, but the protection was associated only to the two major surface glycoproteins (F and G proteins) (Connors et al., 1991). Two different structures of the HRSV F protein: an immature folded form, and other mature cleaved form found in virions have been described (Lawless-Delmedico et al., 2000; López et al., 1998). Although some neutralizing epitopes of the mature form are not found in the immature F protein, both forms induce Ab responses of comparable magnitude (Sakurai et al., 1999). Among different virus isolates, the G protein is the less conserved protein: only a 50% identity for G protein but a 90% for the F protein between the two antigenic subgroups of HRSV (Collins et al., 2007). Therefore, the majority of F-specific but few G-specific monoclonal Abs are cross-reactive (Collins et al., 2007) and thus, very few individual G-protein-specific monoclonal Abs efficiently neutralize HRSV infectivity.

Both immunoglobulin A and G play an important role in protection from HRSV infection. IgA is largely associated with mucosal immunity. As HRSV initially replicates in apical respiratory epithelial cells from the lung airways thus, IgA must be an important factor in protection from initial infection. Fast and specific IgA Ab secretion in the upper airways of primary HRSV-infected mice could be detected (Singleton et al., 2003). Increased infection in human adults correlated with decreased HRSV-specific IgA titers in nasal wash (Walsh &

Falsey, 2004). Also, the intranasal administration of HRSV-specific IgA prior to HRSV infection enhances the antiviral protection compared with untreated mice (Weltzin et al., 1994), although it is not as efficient as administration of HRSV-specific IgG to decrease overall pulmonary viral titers in animals (Fisher et al., 1999). In addition, decreased incidence of severe disease associated with lower-airway infection was found in both elderly and young individuals with high HRSV-neutralizing IgG Ab titers (Falsey & Walsh, 1998; Walsh & Falsey, 2004).

## 3. Cellular immunity

Both experimental models and studies in infected infants indicate the key role of cellular immunity in the control of infection and the virus-induced complex inflammatory processes and airway damage by HRSV. This includes both release of cytokines and chemokines and $CD4^+$ or $CD8^+$ responses.

### 3.1 Cytokines and chemokines

In the primary HRSV infection, the airway epithelial cells and lung-resident leukocytes secrete cytokines and chemokines quickly. These cell-signaling molecules recruit circulating leukocytes to the infected lung that release new cytokines mediating proinflammatory functions. Some of these chemokines and cytokines activate and recruit immune cells. In contrast, others cytokines and chemokines suppress or regulate the proinflammatory state associated to HRSV infection. In summary, this complex cocktail of cytokines, chemokines, and different immune system cells are all implicated in HRSV disease in humans. Even, differences in immunologic responses at birth may be determinant factor of the risk of RSV disease. Comparing cytokines secretion from cord blood samples after stimulation with lipopolysaccharide both healthy control children and hospitalized infants with HRSV, low IL-1β, IL-2, IL-4, IL-5, and IL-10 but high IL-6 and IL-8 responses were found in HRSV-infected young subjects (Juntti et al., 2009). In other studies, also reduced phytohemagglutinin-induced IL-13 response (Gern et al., 2006) and increased IL-4 secretion (Macaubas et al., 2003) were detected in umbilical cord blood cells of infants with risk of severe HRSV infection and asthma. High production of several Th2 cytokines such as IL-4, IL-5, and IL-13 has been associated with severe HRSV airway-disease in infants (Gern et al., 2006). Increased IL-4 was also observed in nasopharyngeal secretions of HRSV-infected children compared to control children (Murai et al., 2007). These results indicate that a Th2 polarization can enhance HRSV-associated illness. In addition, in other study an increased concentration of IL-17 associated to Th17 cells was detected in moderately ill patients as compared with severe HRSV cases but the mechanism was not elucidated (Larranaga et al., 2009). In this line recently, a role of HRSV NS1 protein in the suppression of Th17 response with subsequent decreased protective adaptive immunity had been proposed (Munir et al., 2011).

IFNs are proteins secreted by host cells in response to the presence of viruses among other intracellular pathogens. Different viruses generate IFN-suppressive proteins. HRSV nonstructural proteins NS1 and NS2 cooperatively interfere with the host antiviral cytokine response antagonizing the α/β IFN-induced response in infected epithelial cells as well as suppressing plasmacytoid dendritic cell maturation (Schlender et al., 2000).

### 3.2 T-cell mediated response

Although the immune mechanism involved in HRSV disease and protection is not well understood, humoral and cellular responses appear to play different roles in the antiviral protection, and resolution of HRSV infection as well as disease pathogenesis. The HRSV-specific antibodies are sufficient to prevent or limit the severity of infection but are not required for clearing primary infection (Graham et al., 1991a). However, T cell-mediated responses are necessary to abolish viral replication once HRSV infection is established, and for the clearance of virus-infected cells in primary infection (Anderson & Heilman, 1995).

Studies in mice have shown that F and G envelope proteins prime different subsets of $CD4^+$ T cells. In this system, G protein primes only $CD4^+$ T cells towards a Th2-type cytokine response (Johnson et al., 1998) while F protein primes both $CD4^+$ and $CD8^+$ T cells toward a Th1-type biased cytokine response (Alwan & Openshaw, 1993). In contrast, G protein primes a mixed Th1/Th2 $CD4^+$ T cell response in humans (De Graaff et al., 2004).

Using a murine HRSV-infected model in which the $CD4^+$ and $CD8^+$ T-lymphocyte subpopulations were depleted individually or together by injections of specific Abs, the role of both effector T cells was evaluated. This study showed that both $CD4^+$ and $CD8^+$ lymphocyte subsets are important in clearing a primary infection (Graham et al., 1991b). In addition, $CD4^+$ T cell responses play key roles in pulmonary pathology during infection. IFN-γ secreting $CD4^+$ T cells (Th1-type cytokine response) clear HRSV in low lung pathology, while viral clearance by IL-4 secreting $CD4^+$ cells (Th2-type cytokine response) is strongly associated with significant pulmonary damages, including eosinophilic infiltration (Alwan et al., 1992; Tang & Graham, 1997).

HRSV-specific cytolytic T lymphocytes have been detected in peripheral blood mononuclear cells from infants with bronchiolitis (Chiba et al., 1989; Isaacs et al., 1987). $CD8^+$ T cells with an activated effector cell phenotype could be isolated from bronchoalveolar lavage samples and blood of infants with a severe primary respiratory HRSV infection (Heidema et al., 2007). Although also, the HRSV infection suppresses lung $CD8^+$ T-lymphocyte effector activity by blocking IFN-γ secretion by the HRSV-specific T cells (Chang & Braciale, 2002). In this study, alteration the development of pulmonary CD8+ T-cell memory by interfering with TCR-mediated signaling was described (Chang & Braciale, 2002). Outstandingly, this fault in effector function was only identified in $CD8^+$ T cells from infected lung. In contrast, the effector $CD8^+$ T cells that had migrated from the draining lymph nodes to other secondary lymphoid organs had no insufficiency in their function. Moreover, the viral load after primary HRSV infection was significantly increased in the lungs of IFN-γ knockout mice compared with wild type mice (Lee et al., 2008). Thus, during primary HRSV infection the IFN-γ production is decisive to the development of protection against HRSV-associated disease. The poor induction of IFN-γ release by HRSV may contribute possibly to its weak immunogenicity and the frequent reinfection observed in the human host. In addition, the analysis of responses in congenic mice with different major histocompatibility complex (MHC) haplotypes indicated that susceptibility to sequelae after neonatal HRSV infection was predominantly inherited (Tregoning et al., 2010). Thus, MHC haplotype and its effect on $CD8^+$ T cell immune response play a central role in neonatal HRSV infection. In summary, all these results indicate clearly a key role of $CD8^+$ immune response in virus clearance.

Human cytotoxic T cells from normal not sick adults recognize almost six proteins: F, N, M, M2-1, NS2, and SH but little or no recognition of G, NS1 or P proteins of HRSV was found (Cherrie et al., 1992). As only the recognition of F and M2 proteins were associated with a recent infection, these data indicate the existence of a long term $CD8^+$ response against HRSV (Cherrie et al., 1992). In addition, F, N, and M2 proteins of this virus also induce cytolytic T responses in the mouse model (Kulkarni et al., 1993b),(Olson et al., 2008; Openshaw et al., 1990; Pemberton et al., 1987).

## 4. MHC class I epitopes

In cellular immunity, the recognition and killing of infected cells by $CD8^+$ cytolytic T lymphocytes (CTLs) first requires proteolytic degradation of newly synthesized viral proteins in the cytosol by the combined action of proteasomes and degradative peptidases (Shastri et al., 2002). This antigen processing generates short peptides of 8 to 12 residues that are translocated to the endoplasmic reticulum lumen by transporter associated with antigen processing (TAP), and subsequent N-terminal trimming by the metallo-aminoproteases ERAP1 and 2 is frequently required (Rock et al., 2004; Saveanu et al., 2005). Peptide binding to newly synthesized β2-microglobulin and MHC class I heavy chain generates stable peptide/MHC complexes. Usually, this interaction is made possible by two major anchor residues at position 2 and the C-terminus of the antigenic peptide (Parker et al., 1994; Rammensee et al., 1999) that are deeply accommodated into specific pockets of the antigen recognition site of the MHC class I molecule (Bjorkman et al., 1987a; Bjorkman et al., 1987b). Finally, the stable trimolecular peptide-MHC-$\beta_2$-microglobulin complexes are transported to the cell membrane and presented for CTL recognition (York et al., 1999).

Several HRSV epitopes restricted by different MHC class I molecules of the mouse (H-2) or human (HLA) have been identified using CTL from seropositive individuals or murine models respectively.

### 4.1 H-2 class I epitopes

A study from the early 1990s shown that the cytotoxic activity of lung $CD8^+$ lymphocytes correlated with a protective cellular immune response in BALB/c mice vaccinated with a recombinant virus expressing the M2 protein of HRSV (Kulkarni et al., 1993a). This protein was the only HRSV protein that induced resistance to virus infection in H-$2^d$ mice (BALB/c) but not in H-$2^b$ or H-$2^k$ mice (Kulkarni et al., 1993a). The conserved peptide between subgroup A and B HRSV strains SYIGSINNI, which spans residues 82-90 of the HRSV M2 protein, was identified as the immunodominant $K^d$-restricted CTL epitope in H-$2^d$ mice, and other two M2 peptides were also identified (the different H-2 class I epitopes of HRSV identified are summarized in Table 1)(Kulkarni et al., 1993b). Afterward, a new H-$2K^d$-restricted subdominant epitope with the amino acid sequence VYNTVISYI, spanning residues 127-135 of the same M2 protein, was also identified (Lee et al., 2007). In addition, the frequency of specific T cells responding to two H-$2K^d$-presented epitopes in the same protein following HRSV infection for both lymphoid and nonlymphoid tissues was evaluated. In the cellular response to HRSV infection the ratios of specific T lymphocytes for $M2_{82\text{-}90}$ (dominant) and $M2_{127\text{-}135}$ (subdominant) epitopes were either 3:1 in the spleen or 10:1 in the lung both in primary response, secondary infection, and memory (Lee et al., 2007).

| Strain | H-2 | Sequence [a] | Protein | Position | Reference |
|---|---|---|---|---|---|
| BALB/c | $K^d$ | S<u>Y</u>IGSINN<u>I</u> | Matrix 2 | 82-90 | (Kulkarni et al., 1993b) |
| BALB/c | $K^d$ | E<u>Y</u>ALGVVG<u>V</u> | Matrix 2 | 71-79 | (Kulkarni et al., 1993b) |
| BALB/c | $K^d$ | N<u>Y</u>FEWPPH<u>A</u> | Matrix 2 | 26-34 | (Kulkarni et al., 1993b) |
| BALB/c | $K^d$ | V<u>Y</u>NTVISY<u>I</u> | Matrix 2 | 127-135 | (Lee et al., 2007) |
| BALB/c | $K^d$ | K<u>Y</u>KNAVTE<u>L</u> | Fusion | 85-93 | (Chang et al., 2001) |
| BALB/c | $K^d$ | ELQLLMQSTPPTNNR | Fusion | 92-106 | (Jiang et al., 2002) |
| BALB/c | $K^d$ | T<u>Y</u>MLTNSE<u>L</u> | Fusion | 249-257 | (Johnstone et al., 2004) |
| BALB/c | $K^d$ | T<u>Y</u>MLTNSEL<u>L</u> | Fusion | 249-258 | (Johnstone et al., 2004) |
| C57Bl/6 | $D^b$ | NAIT<u>N</u>AKI<u>I</u> | Matrix | 187-195 | (Rutigliano et al., 2005) |

[a] The H-2$K^d$ or $D^b$ anchor motifs are underlined.

Table 1. Summary of H-2 class I epitopes of HRSV

To identify the epitope(s) responsible to the protective CTL response against HRSV F protein detected in BALB/c mice (Pemberton et al., 1987) a panel of overlapping synthetic peptides corresponding to the complete HRSV F protein sequence of A2 strain was used (Chang et al., 2001). The peptide spanning residues 85-93 of this protein was identified as the immunodominant H-2$K^d$-restricted epitope recognized by F-specific $CD8^+$ T cells from BALB/c mice. No HRSV F-specific CTL response was associated to H-2$D^d$ or H-2$L^d$ class I molecules (Chang et al., 2001).This F-specific $CD8^+$ T cell response was 10-fold lower than the dominant M2-specific response during primary HRSV infection. With the same strategy of screening a panel of overlapping synthetic peptides a second H-2$K^d$-restricted epitope for HRSV F-specific $CD8^+$ CTL was described (Jiang et al., 2002). This is a long 15-mer peptide from the F protein (92–106 residues), which in contrast to other murine HRSV epitopes not contain the known allele-specific motif for H-2$K^d$ binding: Tyr at position 2 (P2) and aliphatic C-terminal residues (Table 1 and SYFPEITHI database: http://www.syfpeithi.de (Rammensee et al., 1999)). All 15 amino acids of this unusual epitope appear to be required for effective presentation to CTL. The interaction of 15-mer epitope with the presenting H-

$2K^d$ class I molecule was not studied. Thus, future studies are needed to clarify this intriguing protein structure.

The use of predictive methods for H-$2^d$ binding by means of web software tools enabled the identification of a new epitope in the F glycoprotein of the Long strain, F249–258, which is presented by $K^d$ as a 9-mer (TYMLTNSEL) or a 10-mer (TYMLTNSELL) peptide (Johnstone et al., 2004). No hierarchy in $CD8^+$ T-lymphocyte responses to F85–93 and F249–258 epitopes was found *in vivo* during a primary response. In contrast, F85–93 was found dominant with respect to F249–258 in *in vivo* memory and secondary responses (Johnstone et al., 2004).

The majority of studies on the HRSV pathogenesis in mice have been performed in the BALB/c mice because are among the most permissive mice but several strains of mice are also susceptible to HRSV infection (Prince et al., 1979). Overlapping synthetic peptide strategy, spanning the F, G, and M proteins of HRSV were used to identify HRSV epitopes in H-$2^b$ mice (Rutigliano et al., 2005). An H-$2D^b$-restricted CTL epitope from the RSV M protein, corresponding to aa 187–195 (NAITNAKII) was identified (Rutigliano et al., 2005). In C57Bl/6 mice, M187–195-specific CTLs were activated with similar kinetics to the immunodominant epitope M2 82–90 in BALB/c mice.

### 4.2 HLA class I epitopes

The peptide $^{308}$NPKASLLSL$^{316}$ was the first human HRSV-specific CTL epitope identified from healthy adult volunteers using a panel of overlapping peptides spanning the HRSV nucleoprotein (Goulder et al., 2000). Later with a similar experimental strategy, other four viral nucleoprotein epitopes were found associated to HLA-A2 (Terrosi et al., 2007) or –B8 (Venter et al., 2003) positive donors (Table 2).

Overlapping peptide approach with the HRSV fusion protein shown the existence of two HLA-B57 and –Cw12-restricted CTL epitopes from infants who had just recovered from severe HRSV infection (Brandenburg et al., 2000). In the same way, HLA-A1-restricted CTL specific for the peptide RELPRFMNYT, spanning residues 109-118 of fusion protein, was detected in peripheral blood mononuclear cells from three healthy volunteers (Rock & Crowe, 2003).

Finally, five epitopes derived from the matrix, NS2 and matrix 2 proteins of HRSV were identified by selection of peptides with appropriate binding motifs, for four different HLA class I alleles (HLA-A1, -A3, -B44 and -B51) using the SYFPEITHI prediction software (Rammensee et al., 1999) from healthy donors (Table 2). All epitopes were detected in 4-5 different individuals suggesting that the dominant epitope was specifically presented by the respective HLA class I allele (Heidema et al., 2004).

Thirteen different HRSV CTL epitopes have been identified either healthy adult donors or sick infants (Table 2). They were presented by three HLA-A, five –B, and one –C class I molecules indicating broad cellular immune control against this virus. All $CD8^+$ epitopes were peptides with the canonical anchor motifs for the respective presenting molecule. The exception was the absence of HLA-A1 anchor motif (Asp or Glu at position 3 and aromatic C-terminal residue) in the ligand $^{109}$RELPRFMNYT$^{118}$ of the fusion protein (Rock & Crowe, 2003), indicating the complexity and plasticity of interactions in HLA-peptide complexes.

In summary, different CTL epitopes have been identified in five of six HRSV proteins previously described as recognized by human $CD8^+$ T lymphocytes by Cherrie et al. (Cherrie et al., 1992). Only the identification of CTL epitopes from SH protein remains open and future studies are needed.

| HLA | Sequence [a] | Protein | Position | Reference |
|---|---|---|---|---|
| A2 | QLLSSSKYT | Nucleoprotein | 16-24 | (Terrosi et al., 2007) |
| A2 | KMLKEMGEV | Nucleoprotein | 137-145 | (Terrosi et al., 2007) |
| B8 | VMLRWGVLA | Nucleoprotein | 258-266 | (Venter et al., 2003) |
| A2 | ILNNPKASL | Nucleoprotein | 303-311 | (Terrosi et al., 2007) |
| B7 | NPKASLLSL | Nucleoprotein | 308-316 | (Goulder et al., 2000) |
| A1 | RELPRFMNYT | Fusion | 109-118 | (Rock & Crowe, 2003) |
| B57 | RARRELPRF | Fusion | 118-126 | (Brandenburg et al., 2000) |
| Cw12 [b] | IAVGLLLYC | Fusion | 551-559 | (Brandenburg et al., 2000) |
| B51 | IPYSGLLLV | Matrix | 195-203 | (Heidema et al., 2004) |
| A1 | YLEKESIYY | Matrix | 229-237 | (Heidema et al., 2004) |
| B44 | AELDRTEEY | Matrix 2 | 64-72 | (Heidema et al., 2004) |
| A3 | RLPADVLKK | Matrix 2 | 151-159 | (Heidema et al., 2004) |
| B51 | LAKAVIHTI | NS2 | 41-49 | (Heidema et al., 2004) |

[a] The HLA anchor motifs are underlined.
[b] No data of HLA-Cw12 anchor motif.

Table 2. Summary of HLA class I epitopes of HRSV

## 5. HLA class I ligands

In most cases, the natural MHC class I ligand is assumed to be the one that has the canonical anchor sites, the minimal length, and the optimal antigenicity when tested as a synthetic peptide although some studies indicates that the extrapolation of either antigenicity or MHC binding strength is not sufficient to identify natural viral MHC class I ligands (Samino et al., 2004; Samino et al., 2006). All previous studies identifying several HRSV epitopes in mice and human were performed with synthetic peptides and no formal identification of natural epitopes was performed. Thus, the high-performance liquid chromatography (HPLC) coupled to high sensitivity mass spectrometry could be a useful technology to identify the endogenously processed HLA ligands derived from HRSV in infected cells.

The basic strategy of this experimental approach beginning with the isolation of HLA-bound peptide repertoires from cells either infected or not infected with HRSV. Next, both peptide pools are fractionated by HPLC in consecutive runs and under identical conditions to reduce alterations in the peptide elution patterns. Later, every HPLC fraction from each peptide pool is analyzed by MALDI-TOF mass spectrometry. Each spectrum of a single HPLC fraction of HRSV-infected cells is compared with the equivalent fraction of the uninfected cells. This technique allows the selection of peptides found only in the HRSV-infected cells. Next, the corresponding MS/MS spectrum of each differential peptide is obtained by ion-trap mass spectrometry and its amino acid sequence is assigned with bioinformatics tools. These sequences could be validated by comparison with the MS/MS spectrum of the corresponding synthetic peptide. With this experimental approach, the viral amino acid sequence AITNAKII, spanning residues 188-195 of the HRSV matrix protein was identified as endogenously processed and presented in infected human cells (Infantes et al., 2011). This natural ligand is a non canonical HLA-Cw4 ligand that uses alternative interactions to the anchor motifs previously described for its presenting HLA-Cw4 class I molecule (Table 3).

Interestingly, the M187-195 NAITNAKII nonamer has been described as an H-2D$^b$-restricted CTL epitope (Rutigliano et al., 2005) in the mouse model (Table 1). It has the canonical anchor motifs for D$^b$ molecules: Asn at position 5 and aliphatic C-terminal residue (Falk et al., 1991). Therefore, two viral peptide species of different lengths that share the same antigenic core and differ only in the additional N-terminal residue were bound to either HLA-Cw4 or H-2D$^b$ presenting molecules in the respective infected cells. Surprisingly, both the human octamer and the mouse nonamer bound efficiently to HLA-Cw4 molecules, in spite of the lack of canonical anchors for interaction with the presenting molecule.

In other study that involved culturing virus-infected cells with stable isotope-labeled amino acids expected to be anchor residues for the HLA allele of interest and then performing immunoprecipitation of HLA molecules and two-dimensional HPLC-mass spectrometry analysis, one HRSV ligand for each HLA-A2 or –B7 class I molecule was identified (Meiring et al., 2006) (Table 3). The ligand KLIHLTNAL (residues 33-41 of the NS1 protein) was presented by HLA-A2 whereas the peptide KARSTPVTL of the fusion protein was endogenously bound to HLA-B7 in human infected cells (Table 3).

Therefore, in the previous studies only one HRSV ligand restricted by a single HLA molecule was presented on the cell membrane surface. Thus, this is the rule? Or conversely, could a particular HLA molecule bind several ligands of this small virus simultaneously? To

answer this question, HLA-B27 ligands isolated from large amounts of human healthy or HRSV-infected cells were compared using mass spectrometry technologies. This analysis demonstrated the existence of nine naturally processed HLA-B27 ligands from six different HRSV proteins in the same infected cells (Infantes et al., 2010) (Table 3). Thus, the nine detected ligands represent 2% of the proteome of this small virus, which is monitored by the same HLA class I allele. If these data are typical for all HLA class I molecules, the cellular immune response would monitor ~ 12% of the proteome of this viral pathogens in heterozygous HLA-A, -B and -C infected individuals. These data suggest that the cellular immune pressure could be high in some small viruses as HRSV.

| HLA | Sequence [a] | Protein | Position | Reference |
|---|---|---|---|---|
| Cw4 | AITNAKII | Matrix | 188-195 | (Infantes et al., 2011) |
| A2 | KLIHLTNAL | NS1 | 33-41 | (Meiring et al., 2006) |
| B7 | KARSTPVTL | Fusion | 551-559 | (Meiring et al., 2006) |
| B27 | HRQDINGKEM | Nucleoprotein | 100-109 | (Infantes et al., 2010) |
| B27 | RRANNVLKNEM | Nucleoprotein | 184-194 | (Infantes et al., 2010) |
| B27 | KRYKGLLPKDI | Nucleoprotein | 195-205 | (Infantes et al., 2010) |
| B27 | SRSALLAQM | Matrix | 76-84 | (Infantes et al., 2010) |
| B27 | VRNKDLNTL | Matrix | 169-177 | (Infantes et al., 2010) |
| B27 | KRLPADVLKK | Matrix 2 | 150-159 | (Infantes et al., 2010) |
| B27 | GRNEVFSNK | Polymerase | 2089-2097 | (Infantes et al., 2010) |
| B27 | LRNEESEKMAK | Phosphoprotein | 198-208 | (Infantes et al., 2010) |
| B27 | HRFIYLINH | NS2 | 37-45 | (Infantes et al., 2010) |

[a] The HLA anchor motifs are underlined.

Table 3. Summary of natural HLA class I ligands of HRSV identified by mass spectrometry.

## 6. Perspectives of HLA-ligand-based vaccine development against HRSV

HRSV lacks effective approved vaccine or antiviral therapy. Worldwide, this virus remains one of the pathogens deemed most important for vaccine development (Hall, 1994). Over the last decades, several efforts have been made towards HRSV vaccine development using different experimental approaches (reviewed in (Murata, 2009)), including inactivated or live attenuated virus strains, vector-based, and viral protein subunit/DNA-based candidates. Also, against pathogens under cellular immune control as HRSV, the polyepitope vaccine approach could be valuable. This vaccine comprises a recombinant DNA construct inserted into a viral genome (generally vaccinia or adenovirus) encoding a chimerical protein where distinct HLA class I-restricted epitopes from one or more pathogens are expressed. Currently, polyepitope vaccine to coinduce multiple CTL responses directed towards a number of different protein target antigens are being used against diseases produced by different pathogens including arenavirus, Epstein Barr virus, HIV, malaria and even several cancers (reviewed in (Suhrbier, 2002)) but not with HRSV. These multiepitope vaccines are thermostable, safe, easy to manufacture, and cost effective. In this context, the identification of pathogen-derived peptides bound to HLA molecules by high resolution mass spectrometry is an emerging focus of HLA-ligand-based vaccine development (Ovsyannikova et al., 2007). In addition, as mutations that enable escape from host cellular immunity against HRSV do not appear to accumulate with time (Collins et al., 2007), thus this virus could be a candidate particularly adequate to the multiepitope vaccine therapeutic approach.

## 7. Conclusion

HRSV is the single most important cause of serious lower respiratory tract illnesses such as bronchiolitis and pneumonia in infants and young children. Mild infections of this virus occur in healthy adults but HRSV poses a serious health risk in immunocompromised individuals and in the elderly. Although the immune mechanisms involved in HRSV disease and protection are not fully understood the $CD8^+$ T lymphocytes are required to clear virus-infected cells. In last decades, several HRSV epitopes restricted by different MHC class I molecules of the mouse (H-2) or human (HLA) have been identified using murine models or CTL of seropositive individuals respectively. In recent years, using mass spectrometry analysis of complex HLA-bound peptide pools, physiologically processed HLA ligands from HRSV have been identified. This knowledge could be used in the peptide-based vaccine development.

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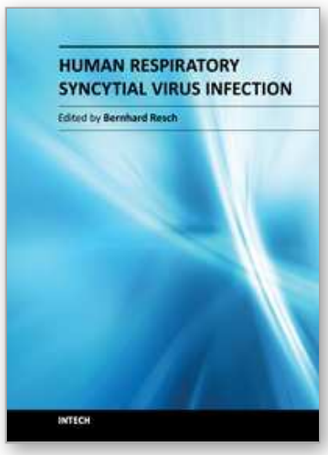


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In this online Open Access book on "Human RSV Infections", several distinguished authors contribute their experience in respiratory syncytial virology. A major focus lies on the fascinating pathophysiology of RSV and represents recent and actual work on different mechanisms involved in the complex pathogenesis of the virus. The second section elucidates epidemiologic and diagnostic aspects of RSV infection covering a more clinical view of RSV disease. At last, treatment modalities including the search for a vaccine that is still not in sight are discussed and conclude this book, thus building up a circle that runs from experimental models of RSV related lung disease over clinical aspects of disease to the latest news of therapeutic and prophylactic approaches to human RSV infection.



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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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