MHC class I - Peptide binding and complex stability

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MHC class I

- Peptide binding and complex stability

Elna Follin

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Department of Pathology, University of Cambridge

With the approval of the Lund University Faculty of Medicine,
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MHC class I – Peptide binding and complex stability

Abstract
The major histocompatibility class I (MHC-I) genes are highly polymorphic and the proteins that they encode play a crucial role in both the innate and the adaptive immune response. A MHC-I molecule consists of three parts: one polymorphic heavy chain, one invariant light chain, β2-microglobulin and a peptide of usually between 8-11 amino acids in length. The maturation and quality control of MHC-I takes place in the endoplasmic reticulum and involves several different proteins including the MHC-I dedicated protein tapasin.

In this thesis we have studied different parameters important for MHC-I formation and stability in humans and birds. We have used various approaches including in silico prediction methods, biochemical assays and cellular assays to elucidate the MHC-I maturation. We show that the functional relationships between MHC-I molecules in passerine birds of different species are based on the MHC-I characteristics such as peptide-binding specificity rather than species characteristics. In addition, passerine MHC-I molecules similar to human MHC-I molecules, have a complex dissociation. This suggests that just as in humans, passerine MHC-I molecules go through different maturation stages that most likely include interaction with quality control proteins such as tapasin. The cell surface expression of stable MHC-I molecules is crucial for the function of the adaptive immune response and for this reason MHC-I and its related proteins are often a target for viral and tumour evasion strategies. In human cells we show that tapasin promotes the formation of stable cell surface expressed MHC-I molecules and that the dependency on tapasin for a stable cell surface expression varies between different allomorphic (allele specific protein produces). The dysregulation of tapasin results in alterations in the peptide repertoire that is presented by MHC-I at the cell surface and most often this induces a decreased stability of the expressed molecules. We here show that by adding certain peptides exogenously to cells deficient in tapasin we were able to increase MHC-I cell surface stability significantly suggesting that exogenous modulations of tapasin deficient cells might be a possible approach in immunotherapy. The formation of aberrant conformations of HLA-B*27:05 has been suggested to play a role in the pathogenesis of ankylosing spondylitis and here we showed that tapasin has a preventive effect on the formation and presentation of aberrant conformations of HLA-B*27:05 at the cell surface.

In conclusion we show that the complex kinetics of MHC-I maturation and stability is a trait shared between birds and humans and we suggest that by studying MHC-I in other species than human we can gain valuable insight into the complex world of MHC-I.

Key words
MHC-I, HLA-I, Tapasin, Passerine bird, Stability, Antigen, Peptide, Homodimer

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Date 2014-02-21
MHC class I

- Peptide binding and complex stability

Elna Follin
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MHC-I and disease

Tumour immune evasion strategies

Viral immune evasion strategies

Strategies targeting TAP

Strategies targeting Tapasin

HLA-I and disease association

Ankylosing spondylitis

Immunotherapy and MHC-I antigen presentation

DISCUSSION OF THE ARTICLES

Paper I

Paper II

Paper III

Paper IV

POPULÄRVETENSKAPLIG SAMMANFATTNING

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REFERENCES

APPENDIX (PAPERS I-IV)
PAPERS INCLUDED IN THE THESIS

I. **In silico peptide-binding predictions of passerine MHC class I reveal similarities across distantly related species, suggesting convergence on the level of protein function**

Elna Follin, Maria Karlsson, Claus Lundegaard, Morten Nielsen, Stefan Wallin, Kajsa Paulsson*, Helena Westerdahl#

*Kajsa Paulsson and Helena Westerdahl contributed equally to this work.

Immunogenetics 65:299-311 2013

II. **Great reed warbler major histocompatibility complex class I molecules with considerable differences on the level of amino acids share peptide preferences**

Elna Follin, Michael Rasmussen, Maria Strandh, Søren Buus, Morten Nielsen, Helena Westerdahl*, Kajsa M. Paulsson#

*Kajsa Paulsson and Helena Westerdahl contributed equally to this work.

Manuscript

III. **Stability and conformation of HLA-I allomorphs are influenced by tapasin**

Victoria Junghans, Elna Follin, Oliwia Rysnik, Michael Rasmussen, Søren Buus, Paul Bowness and Kajsa M. Paulsson

Manuscript

IV. **Modulation of HLA class I on the surface of tapasin deficient cells**

Elna Follin, Victoria Junghans, Gustav Røder, Kajsa M. Paulsson

Manuscript
Cytotoxic effect of menadione and sodium orthovanadate in combination on human glioma cells

Zahid M. Delwar, Dimitrios Avramidis, Elna Follin, Yan Hua and Åke Siden, Mabel Cruz, Kajsa M. Paulsson and Juan Sebastian Yakisich

Investigational New Drugs, 2012, Volume 30, Number 4, Pages 1302–1310

Histocompatibility, Chapter 10, MHC Class I Quality Control

Gustav Røder, Linda Geironson, Elna Follin, Camilla Thuring and Kajsa Paulsson


Glioblastoma cell lines differ in tapasin dependence of HLA-I genotype profiles and have extensive down-regulation of antigen processing machinery components and MHC-I cell surface expression

Camilla Thuring, Linda Geironson, Elna Follin, Edward Visse, Michael Rasmussen, Mikkel Harndahl, Søren Buus, Gustav Røder and Kajsa Paulsson

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<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>APM</td>
<td>antigen processing machinery</td>
</tr>
<tr>
<td>AS</td>
<td>ankylosing spondylitis</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine tri-phosphate</td>
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<tr>
<td>BFA</td>
<td>brefeldin A</td>
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<tr>
<td>β2m</td>
<td>β2-microglobulin</td>
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<tr>
<td>Cnx</td>
<td>calnexin</td>
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<tr>
<td>COP</td>
<td>coat protein complex</td>
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<tr>
<td>Crt</td>
<td>calreticulin</td>
</tr>
<tr>
<td>DRiPs</td>
<td>defective ribosomal products</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>ERAAP</td>
<td>ER aminopeptidase associated with antigen processing</td>
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<tr>
<td>HC</td>
<td>heavy chain</td>
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<tr>
<td>HCMV</td>
<td>human cytomegalovirus</td>
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<td>HLA</td>
<td>human leucocyte antigen</td>
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<tr>
<td>HSV</td>
<td>herpes simplex virus</td>
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<tr>
<td>IC47</td>
<td>infected cell protein 47</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>ISRE</td>
<td>interferon stimulated responsive element</td>
</tr>
<tr>
<td>LMP</td>
<td>low molecular weight protein</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>pHLA-I</td>
<td>peptide human leucocyte antigen class I</td>
</tr>
<tr>
<td>PLC</td>
<td>peptide loading complex</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>pMHC-I</td>
<td>peptide-major histocompatibility complex class I</td>
</tr>
<tr>
<td>PSCPL</td>
<td>positional scanning combinatorial peptide library</td>
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<tr>
<td>SPA</td>
<td>scintillation proximity assay</td>
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<tr>
<td>Tapasin</td>
<td>TAP-associated glycoprotein</td>
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<tr>
<td>TAP</td>
<td>transporter associated with antigen processing</td>
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<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>Tpn</td>
<td>tapasin</td>
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<tr>
<td>UPR</td>
<td>unfolded protein response</td>
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The main function of the immune system is to defend the body against harmful invading pathogens such as bacteria, fungi, parasites and viruses as well as to inhibit tumour formation. The immune system is classically divided into two major systems, the innate and the adaptive. The innate immune system is what one would call the first line of defence and is immediate and non-specific. This part of the immune system is made up by physical barriers, chemical factors and cellular responses. If the innate immune system is compromised or breached the adaptive immune system is activated. In contrast to the innate immune system, which responds in a non-specific manner, the adaptive immune response is antigen specific and results in immunological memory. Given that my main focus is antigen presentation I will concentrate on the adaptive immune system from here on.

In the adaptive immune system we find two major groups of effector cells, the B lymphocytes (B cells) and the T lymphocytes (T cells). These two cell types exert their effect in different ways. After activation B cells produce antibodies that bind and contribute to the elimination of extracellular antigens. In contrast, activated T cells can only recognise surface expressed antigens that are presented in the context of major histocompatibility complex (MHC). MHC is found in two forms, the MHC class I (MHC-I) and the MHC class II (MHC-II).
The peptides presented by MHC-I are mainly generated from intracellular self-proteins and pathogens such as viruses but also peptides from tumour proteins. The MHC-II present peptides generated from extracellular proteins and pathogens such as bacteria. The MHC-I molecules present antigens to T cells carrying the co-receptor CD8 (CD8+ T cells). The CD8+ T cells, or the cytotoxic T cells as they are also called, recognise MHC-I molecules presenting peptides through their receptors (TCRs). If the peptide presented by MHC-I indicates infection or tumour formation, the cytotoxic T cell will kill the damaged cell. The MHC-II molecules present peptides to T cells carrying the co-receptor CD4 (CD4+ T cells). The CD4+ T cells, or the T helper cells as they are also called, bind and recognise peptide-presenting MHC-II molecules. When a MHC-II molecule presents a pathogenic peptide the CD4+ T cells will produce and secrete chemical factors resulting in the activation of mechanisms that subsequently will eliminate the source of infection.

A functional antigen presentation is of crucial importance for the function of our immune system. During optimal conditions, the presence of pathogens and different malignancies is exposed and removed from the body before disease can develop. However, if antigen presentation is suppressed or absent, cells infected with pathogens or tumour cells will not be recognised by the body as harmful, resulting in the development of infection and cancer. The main focus during my PhD has been to elucidate the function of MHC-I antigen presentation in both birds and humans and also to study the influence of peptide selection on molecular stability.
MHC CLASS I MATURATION AND FUNCTION

MHC-I gene organisation and polymorphism

The MHC genes were originally discovered because of the role the proteins they encode play in the rejection of transplants made between MHC incompatible mice. The studies resulted in the finding of the mouse MHC-I genes H-2K, H-2D and H-2L. The human MHC region is large, with over 200 genes spanning four mega bases. The region is found on the short arm of chromosome six. The human version of MHC is called human leucocyte antigen (HLA). The HLA class I (HLA-I) genes are divided into two groups, the classical and the non-classical molecules. The classical HLA-I genes, characterised by high sequence polymorphism and their ability to bind antigenic peptides and induce graft rejection, are encoded by three different genes; the HLA-A, -B and -C. The HLA-A, -B and -C are co-dominantly expressed which means that each individual can express between three and six different classical HLA-I molecules. The non-classical HLA-I genes, are characterised by (as compared to the classical HLA-I genes) low sequence polymorphism, tissue specific expression, lower cell surface expression and binding of both lipids and peptides. In addition, some of these molecules have been suggested to be expressed at the cell surface as empty molecules.

It is well known that the HLA-I genes are the most polymorphic genes found in the human genome with over 7900 different alleles known to date (January 2014). 98% of these are made up by the classical HLA-A, -B and -C alleles.
Among the HLA-I genes the most polymorphic are the HLA-B, making up more than 40% of the alleles. The HLA-A makes up more than 30% and the HLA-C is responsible for around 27% of the alleles known to date (http://hla.alleles.org/nomenclature/stats.html). It is worth noting that not all of these alleles are translated into proteins with unique function. This is because all DNA sequence differences do not result in differences in amino acid sequence. The 7900 different alleles known, give rise to approximately 5800 different proteins or allomorphs (allele specific protein products) with HLA-B proteins being responsible for 42%, HLA-A for 32% and HLA-C for 26% (http://hla.alleles.org/nomenclature/stats.html).

**MHC-I structure and peptide-binding**

The MHC-I molecule is believed to consist of two polypeptide chains, the polymorphic heavy chain (HC), and the invariant light chain β₂-microglobulin (β₂m). However, for the molecule to reach the correct and mature conformation that is presented in a stable form at the cell surface, an optimal peptide needs to be bound and included in this structure. The traditional description of a MHC-I molecule is a heterodimer, however, studies have revealed that other conformations might also exist for certain allomorphs e.g., of the HLA-B*27 supertype family (Allen et al. 1999; Mear et al. 1999). This will be discussed further in the passage on HLA-B*27:05 and ankylosing spondylitis (AS).
In a mature MHC-I molecule the HC is folded into three domains, the \( \alpha_1 \), the \( \alpha_2 \) and the \( \alpha_3 \). During folding of the MHC-I HC, disulphide bonds are formed; one within the \( \alpha_3 \) domain, between Cys203 and Cys259, and one within the \( \alpha_2 \) domain between Cys101 and Cys164 (Ferre et al. 2003; Tector et al. 1997). The \( \alpha_3 \) domain, which is folded into an immunoglobulin-like conformation that is anchored to the cell via a trans membrane region that continues into the cytoplasm, is non-covalently bound to \( \beta_2 \text{m} \) (Bjorkman et al. 1987). \( \beta_2 \text{m} \) is encoded on chromosome 15 (Goodfellow et al. 1975).

The \( \alpha_3 \) domain is less polymorphic than the two other domains. It is to this domain as well as to the \( \alpha_2 \) domain that the T cell co-receptor CD8 binds during the TCR-MHC-I interaction (Gao et al. 1997). In a mature, cell surface expressed, MHC-I molecule a peptide is bound in the peptide-binding groove. This groove is made up of the \( \alpha_1 \) and \( \alpha_2 \) domains of the HC and it is, not surprisingly, in this peptide-binding groove that the majority of the MHC-I polymorphism resides (Bjorkman et al. 1987). The optimal length for peptides bound in the peptide-binding groove is suggested to be 8-10 amino acids (Rammensee et al. 1995). However, more recent studies have shown that also longer peptides are able to bind and form stable complexes (Bell et al. 2009; Ebert et al. 2009; Geironson et al. 2013; Probst-Kepper et al. 2001).

The peptide is bound in the peptide-binding groove by both sequence-dependent and sequence-independent interactions (Praveen et al. 2010).
The latter interactions are formed through hydrogen bonds between the N- and C-terminal parts of the peptide and conserved amino acids in the MHC-I peptide-binding groove (Praveen et al. 2010). The sequence-dependent interactions are formed through non-covalent bonds between the peptide side-chains and the binding pockets that make up the peptide-binding groove (Praveen et al. 2010). The amino acid preferences of these pockets differ between different allomorphs. The unique peptide-binding preference of an allomorph can be displayed using a peptide-binding motif. The motif shows which positions in the peptide that are important for binding to the MHC-I molecule and these are termed anchor positions. In addition, other positions of the peptide that influence the peptide-binding are also shown (see figure 1). Although, it has been shown that several MHC-I allomorphs display anchor position at position two and at the C-terminal of the peptide, the peptide-binding preference can be markedly different between different allomorphs. This is what we here refer to as allomorph peptide-binding specificity (Lund et al. 2004).
Figure 1: Peptide-binding motif of HLA-A*02:01. The red arrows mark the two anchor positions of the peptide, which are essential for binding of the peptide in the MHC-I peptide-binding groove. The blue arrows mark other positions of the peptide that influence the quality of the binding.

MHC-I maturation

The maturation of MHC-I is a sequential and complex process involving several different proteins that takes place in the endoplasmic reticulum (ER) (see figure 2). Decaying proteins or defective ribosomal products (DRiPs), proteins that are degraded before they have reached a correct conformation, as well as longer polypeptides are first degraded into shorter peptides by the proteasome in the cytosol. They are then transported via the transporter associated with antigen processing (TAP) into the ER lumen (Abele and Tampe 2009; Cascio et al. 2001).
The peptides that reach the ER can be further trimmed by the aminopeptidases ERAP1 and ERAP2 before they are suitable for binding to MHC-I molecules (Saric et al. 2002; Saveanu et al. 2005). The MHC-I HC is translated directly into the ER lumen where the membrane bound chaperone protein, calnexin (Cnx) helps in the initial folding and stabilises the HC until β2m is bound (Nossner and Parham 1995). Cnx also recruits the thiol-oxidoreductase ERp57 that has been shown to be involved in disulphide bond formation (Oliver et al. 1999; Zhang et al. 2006). β2m is then subsequently bound to the MHC-I HC and Cnx is replaced by its soluble homolog calreticulin (Crt) (Sadasivan et al. 1996). After the binding of β2m to the MHC-I HC, some molecules can bind peptides without further help and progress out to the cell surface for presentation with sufficient stability. However, most HC-β2m molecules are in need of assistance to bind optimal peptides and are thus recruited into the so-called peptide loading complex (PLC). The PLC consists of Crt, ERp57, TAP and tapasin (Cresswell et al. 1999; Hughes and Cresswell 1998; Ortmann et al. 1994; Ortmann et al. 1997; Suh et al. 1994). After the MHC-I molecule has been loaded with an optimal peptide, the molecule is released from the PLC and is free to progress out to the cell surface for presentation to patrolling cytotoxic T cells.
Figure 2: Sequential maturation of MHC-I within the ER. The MHC-I HC is first translated on the ribosome and co-translationally inserted into the ER lumen where the membrane bound chaperone Cnx aids in the initial folding of the MHC-I HC. β2m then binds to the MHC-I HC and Cnx is replaced by Crt. At this point some MHC-I molecules might bind peptide and progress to the cell surface. Molecules that do not progress to the cell surface are incorporated into the PLC with Crt, TAP, tapasin and ERp57 for loading of optimal peptides.
Transport of peptides into the ER

The function of TAP is to translocate peptides from the cytosol into the ER lumen for potential presentation by MHC-I. The TAP1 and TAP2 genes are found within the MHC-II region in humans, next to the genes encoding the two immunoproteasome subunits low molecular weight protein (LMP) 2 and LMP7 (McCluskey et al. 2004). Based on studies that have shown a shared preference for proteasomal generation of peptides (proteasome), peptide translocation (TAP) and peptide-binding (MHC-I) it has been suggested that genes encoding these functional proteins have coevolved to ensure optimal antigen presentation (McCluskey et al. 2004; Nielsen et al. 2005). One hypothesis is that the immunoproteasome subunits and TAP has coevolved to customise peptide specificity for specific MHC-I allomorphs, however this has not been proven in the human system. Evidence instead suggests that human TAP binds and translocates peptides that are generally well suited for binding to a wide array of MHC-I molecules indicating that the coevolution between MHC-I and TAP genes is not linkage dependent (Obst et al. 1995). Studies on TAP polymorphism have found only minor differences between different allomorphs of the protein suggesting that the functional polymorphism of TAP is low (McCluskey et al. 2004; Obst et al. 1995).

TAP is a heterodimer that consist of the TAP1 and TAP2 proteins. The expression of these proteins can be up-regulated by interferon-γ (IFN-γ) (Epperson et al. 1992; Seliger et al. 1997). These proteins are members of the adenosine tri-phosphate (ATP) binding cassette family of transporter
molecules, in which members contain N-terminal membrane spanning segments and a C-terminal cytoplasmic nucleotide-binding domain (Gaudet and Wiley 2001). The TAP1 and TAP2 proteins together form a channel between the cell cytosol and the ER lumen and it is through this channel that the peptides are actively transported. The transport itself occurs in a two-step process. The first step, when the peptide binds to TAP, is ATP-independent and the second step, involving the translocation of the peptide from the cytosol into the ER lumen is ATP-dependent (Androlewicz et al. 1993; Neefjes et al. 1993). It has been shown that peptides of 8-16 amino acids in length are most efficiently translocated by the human TAP heterodimer (van Endert et al. 1994). TAP is an important part of the PLC and it has been shown that the MHC-I expression of TAP-deficient cells is greatly impaired and the MHC-I molecules that are expressed are unstable and rapidly degraded (Salter and Cresswell 1986; Spies et al. 1992; Spies and DeMars 1991).

Trimming of peptides inside the ER

After peptides have been translocated via TAP into the ER, they may need to be further trimmed to become optimal epitopes for MHC-I binding. This trimming is accomplished by the ER aminopeptidase associated with antigen processing (ERAAP in mice and ERAP1 and ERAP2 in human) (Saric et al. 2002; Saveanu et al. 2005; Serwold et al. 2002). Both ERAP1 and ERAP2 remove N-terminal extensions of peptides and they have been shown to have a preference for peptides with 10 amino acids or longer in length (Serwold et al.
Although, ERAP1 and ERAP2 both trim peptides at the N-terminus they bind peptides with different C-terminal residues. ERAP1 binds peptides with large hydrophobic C-terminals and ERAP2 prefers peptides with basic C-terminals (Saveanu et al. 2005). The genes that encode for ERAP are both found outside the MHC region and the protein expression is up-regulated as a response to IFN-γ (Serwold et al. 2002).

**MHC-I quality control**

TAP-associated glycoprotein (tapasin) is a MHC-I devoted protein dedicated to MHC-I quality control. Tapasin is a 48 kD type 1 transmembrane protein and consists of three parts, a N-terminal ER luminal region, a transmembrane-spanning region and a small cytoplasmic tail (Li et al. 1997; Ortmann et al. 1997). The N-terminal ER luminal region can be further divided into two domains, domain-1 spanning the first 1-269 amino acids and domain-2 spanning residues 270-381 (Dong et al. 2009). The human tapasin gene is found within the MHC-II region on chromosome six and is evolutionary well conserved (Herberg et al. 1998). Within the human population, only a few different tapasin alleles have been described, however no functional differences have been observed (Copeman et al. 1998; Furukawa et al. 1998).

Several sites of interaction between tapasin and MHC-I have been suggested and they are all found within the ER luminal domain of tapasin (Carreno et al. 1995; Dong et al. 2009; Lewis et al. 1996; Peace-Brewer et al. 1996; Yu et al. 1999).
**Tapasin interaction with TAP**

Tapasin is, as previously stated, involved in MHC-I quality control where it plays an important role through several modes of action. In the PLC, the transmembrane part of tapasin binds to TAP and forms a bridge between the MHC-I molecule and TAP thereby putting the peptide receptive MHC-I molecule in close proximity to the peptides translocated by TAP (Koch et al. 2004; Koch et al. 2006; Li et al. 1997; Ortmann et al. 1997). By binding to TAP, tapasin also stabilises the TAP heterodimer and promotes the binding of peptides to TAP, which then results in an increase of peptides translocated into the ER lumen (Garbi et al. 2003; Lehner et al. 1998; Li et al. 2000; Tan et al. 2002).

**Peptide editing**

Tapasin has been suggested to stabilise immature MHC-I molecules in the ER, and keeping them in a peptide-receptive state (Chen and Bouvier 2007; Garstka et al. 2011; Lehner et al. 1998; Ortmann et al. 1997). Tapasin has also been shown to be involved in shaping the peptide repertoire presented by MHC-I. It has been suggested that this is accomplished by tapasin’s involvement in a process called peptide editing or peptide optimisation, which essentially means the exchange of bound low affinity peptides to peptides with higher affinity and/or replacement of peptides inducing a less stable conformation for peptides inducing a more stable peptide-MHC-I (pMHC-I) complex (Howarth et al. 2004; Praveen et al. 2010; Wearsch and Cresswell 2007; Williams et al. 2002). Hence, peptide affinity alone does not determine
the selection of peptide and the peptide repertoire presented by MHC-I. Surface stability of the pMHC-I complex has indeed been shown to be of great importance (Howarth et al. 2004). In a study performed by Howarth and colleagues a set of peptides with different abilities to induce stable pMHC-I complexes were introduced as mini-genes into two cell lines, the tapasin deficient 721.220Kb and the tapasin proficient 721.220Kb tapasin, to study the influence of tapasin on the cell surface expressed MHC-I molecules and their peptide cargo. This study showed that in the presence of tapasin a hierarchy was established based on pMHC-I complex half-life, with the peptides inducing the longest half-lives of pMHC-I being presented to the highest degree (Howarth et al. 2004). In contrast, in the absence of tapasin this hierarchy was lost and the peptides able to induce pMHC-I complexes with intermediate half-lives formed the most complexes with MHC-I. The peptides used in the study showed only small difference in their ability to bind to MHC-I, which would suggest that the peptide hierarchy observed in the presence of tapasin is most likely formed based on pMHC-I complex half-life rather then the peptide affinity per se (Howarth et al. 2004). In addition it has also been shown using in vivo systems that tapasin is able to establish peptide hierarchies according to the pMHC-I complex stability with corresponding responses by CD8+ T cells (Thirdborough et al. 2008). Interestingly, in a study by Zarling and colleagues where the influence of tapasin on HLA-B8 was studied it was shown that the average affinity of the peptide pools eluted from cells either proficient or deficient in tapasin was not significantly different (Zarling et al. 2003). However, they also show that the pMHC-I complex half-life is higher in the presence of tapasin, this was shown for both
HLA-B8 and HLA-A*02:01. In light of the results from Howarth and colleagues one can conclude that tapasin can edit the peptide repertoire not only based on affinity but also based on stability. This is also in line with other studies suggesting that both affinity and stability are important factors in the peptide editing process (Chen and Bouvier 2007; Wearsch and Cresswell 2007).

The thiol-disulfide oxidoreductase ERp57 has been shown to interact with tapasin in the PLC (Dick et al. 2002). The role that ERp57 plays in maturation of MHC-I is debated. It has been shown that in a cell-free system a conjugate of ERp57 and soluble tapasin more efficiently than soluble tapasin alone stabilised empty MHC-I molecules and selectively facilitated binding of high affinity peptides thereby promoting peptide editing (Wearsch and Cresswell 2007). This suggests that ERp57 might play a structural role in the PLC promoting the effects of tapasin.

Tapasin contains a C-terminal motif (KKXX) that has been shown to function as an ER retrieval signal in combination with the coat protein complex I (COPI) coatomer (Cosson and Letourneur 1994; Paulsson et al. 2002). Interestingly, in a study by Paulsson and colleagues, removal of this ER retrieval signal through mutation of the C-terminal motif of tapasin, increased the amount of cell surface expressed MHC-I molecules, the molecules were however shown to be of worse quality with a higher degradation rate as compared to in cells with wild type tapasin (Paulsson et al. 2006).
This indicates that in addition to the events taking place within the PLC, tapasin is also promoting peptide editing through recycling of immature MHC-I molecules. Others have also shown the important role that tapasin plays in retention and recycling of immature and sub-optimally loaded MHC-I molecules (Bresnahan et al. 1997; Grandea et al. 2000; Hsu et al. 1991; Park et al. 2001; Paulsson et al. 2002; Schoenhals et al. 1999).

**Tapasin dependence and stability**

The tapasin dependence of an allomorph is defined as the dependence on tapasin for stable cell surface presentation of HLA-I and allomorphs differ greatly in their dependence on tapasin. Some allomorphs e.g., HLA-B*44:02 exhibit very low cell surface expression in the absence of tapasin while other allomorphs, such as HLA-B*27:05, retain a high cell surface expression even in the absence of tapasin (Peh et al. 1998). Recent work from our group using a biochemical approach with recombinant proteins to study the ability to form pMHC-I complexes in the presence and absence of tapasin, revealed that the level of tapasin facilitation varied greatly for the 16 HLA-A and -B allomorphs studied (Geironson et al. 2013). Interestingly, the tapasin facilitation hierarchy described correlated very well with previous cellular studies on tapasin dependency (Park et al. 2003; Peh et al. 1998).

Studies have suggested that several specific amino acids at defined positions in the MHC-I protein might be of importance for the different degrees of tapasin dependence (Garstka et al. 2011; Park et al. 2003).
It has been suggested that the more tapasin dependent allomorphs might exhibit a conformational disordered state, which obstructs the binding of peptide in the absence of tapasin. When tapasin interacts with these molecules a more ordered conformation is achieved that allows for optimal peptide-binding (Garstka et al. 2011). Several amino acid positions, including 114 and 116 of the MHC-I protein have been suggested to be responsible for these different conformational states (Garstka et al. 2011; Park et al. 2003).

Tapasin does not only affect the cell surface stability of pMHC-I complexes. Studies of the HLA-B*27:05 allomorph, shown to be low in tapasin dependency, have revealed that the peptides presented in the presence of tapasin, significantly differ from the peptides presented in its absence (Peh et al. 1998; Purcell et al. 2001). Interestingly, when the level of cytotoxicity was measured four hours post infection with recombinant vaccinia virus expressing defined Epstein-Barr virus antigens, cells proficient in tapasin were more efficiently killed as compared to cells deficient in tapasin. This was shown for both the HLA-B8 and the HLA-B*27:05 allomorph (Peh et al. 1998). These results raise the question if tapasin dependence should be defined only by the cell surface stability of the MHC-I molecules or if the shaping of the peptide repertoire also should be taken into account?
MHC-I in birds and the importance to study MHC-I in other species than human

The MHC genes are among the most extensively investigated to date (2014) and several animals species have been studied in an attempt to better understand the function and dysfunction of these genes and their protein products. From the beginning, most MHC studies in animals were done for economical reasons, to be able to take preventive measures in breeding to avoid certain infections and diseases. In later years it has become more apparent that the function and build-up of the immune system in different species may differ significantly but that the aim, fighting of infection is the same and that the use of comparative studies may prove useful in understanding the function of the human system. In addition, numerous discoveries first made in animals, such as the discovery of MHC-I and MHC-II, were later also discovered in humans after performing comparative studies. To further our understanding of the function of MHC it is also important to understand its origin and evolution by investigating these genes and their protein products in different species. Comparative studies may also be beneficial in prevention/treatment of infections and diseases that could be transmitted between humans and animals (zoonoses). Mammals such as human, mouse and rat are the most well-studied species in regards of MHC genes but also non-mammals such as birds have been extensively studied.
Avian MHC-I gene organisation and protein expression

The classical chicken major histocompatibility complex (MHC) region, also named the B locus or MHC-B, is simple and small with 19 genes, two of which are classical MHC-I genes, spanning over 92 kb (Guillemot et al. 1988; Kaufman et al. 1999). This can be compared to the approximately 200 genes spanning four mega bases in the human MHC region. The classical chicken MHC region is found on chromosome 16 and the β₂m is found on chromosome ten (Bloom et al. 1987; Riegert et al. 1996). In addition, a MHC-Y region has been found in chicken that encode both MHC-I and MHC-II β genes however these genes exhibit low polymorphism, low sequence divergence, low expression levels and does not show a strong effect on graft rejection, mixed lymphocyte reaction and disease resistance leading to the conclusion that these genes are non-classical (JuulMadsen et al. 1997; Pharr et al. 1996; Vallejo et al. 1997; Zoorob et al. 1993).

The small size and the compact gene organisation of the MHC-B region is the reason behind the term “minimal essential MHC” (Kaufman et al. 1999; Kaufman et al. 1995). In comparison, studies in other birds of the order Galliformes, such as the turkey (Meleagris gallopavo), the quail (Coturnix japonica), the black grouse (Tetrao tetrix) and the golden pheasant (Chrysolophus pictus), all have revealed a, similar to the chicken, small and simple MHC organisation with few MHC-I genes e.g., black grouse and golden pheasant has two MHC-I genes, turkey has three MHC-I genes but quails has as many as seven (Chaves et al. 2009; Guillemot et al. 1988;
Kaufman et al. 1999; Shiina et al. 2004; Wang et al. 2012; Ye et al. 2012). Interestingly, only one out of the two MHC-I genes found in the chicken is highly transcribed and dominantly expressed at the cell surface and the quail show a higher transcription for only two of their seven MHC-I genes (Kaufman et al. 1995; Shaw et al. 2007; Shiina et al. 2004; Wallny et al. 2006). In addition other bird orders such as Anseriformes, with the mallard duck (*Anas platyrhynchos*) have been shown to have five MHC-I genes however only one, which is highly transcribed (Moon et al. 2005).

It was long believed that the simple MHC organisation of the chicken was applicable for all birds however studies in other birds i.e., songbirds of the order Passeriformes have revealed that this is not the case. Although the understanding of the MHC organisation in songbirds is still largely fragmented, studies in a small songbird, the zebra finch (*Taeniopygia guttata*), has shown that MHC genes, MHC-B, that has been shown to be linked in the chicken are dispersed over at least two or maybe even four different chromosomes in the zebra finch (Balakrishnan et al. 2010; Ekblom et al. 2011). Numerous studies also reveal a higher number of functional MHC-I genes in songbirds as compared to both chicken and other species of the order Galliformes (Bonneaud et al. 2004; Promerova et al. 2012; Schut et al. 2011; Sepil et al. 2012; Westerdahl 2007; Westerdahl et al. 1999; Wittzell et al. 1998). The studies performed in songbirds has so far not been able to assign the different MHC-I alleles to specific loci and very little is known about the transcription level of these genes as well as their protein expression and function (Karlsson and Westerdahl 2013; Sepil et al. 2012; Westerdahl et al. 1998).
One could speculate that the high number of MHC-I genes found in songbirds results from that each MHC-I has a very narrow peptide-binding preference and thus many genes are needed for an optimal antigen presentation that is able to present a wide range of pathogenic peptides. In contrast, one could also speculate that, like in the chicken, only few of these genes are highly transcribed and expressed as proteins and that the ones that are expressed as proteins have broader peptide-binding preference. However, without functional studies on MHC-I protein expression all we can do is speculate.

Several studies have shown strong associations between specific MHC-I allomorphs and resistance or susceptibility to certain infectious diseases such as Raus sarcoma virus and Mareks disease in chicken. It has been proposed that in these birds one reason for these associations could be the single dominantly expressed MHC-I molecule (Briles et al. 1977; Kaufman et al. 1999; Kaufman et al. 1995; Wallny et al. 2006). Based on this one could speculate that a higher number of MHC-I genes per individual would be beneficial as the number of MHC-I molecules expressed would increase, which would result in the presentation of a broader peptide repertoire. The presentation of a broader peptide repertoire would most likely facilitate the recognition of a potential pathogen to a higher degree than if only one single specificity molecule would be responsible for the surface presentation. In line with this reasoning, studies on songbirds that have been shown to have a higher number of functional MHC-I genes show some associations between specific MHC-I alleles and resistance but also susceptibility to malaria infections (Bonneaud et
al. 2006; Loiseau et al. 2008; Westerdahl et al. 2012; Westerdahl et al. 2013). However, it is important to note that these studies are correlative and based on the presence of different MHC-I alleles in populations of birds that has been shown to be either resistant or susceptible to infection. For this reason it is important to perform studies on these birds where the actual MHC-I protein expression can be studied to be able to draw any firm conclusions on resistance and susceptibility to different infectious diseases.

_TAP_

In the human genome the TAP genes are found within the MHC class II region flanking the LMP2 and LMP7 genes (McCluskey et al. 2004). In contrast, the TAP genes, of birds of the order Galliformes are localised either close to or between the two MHC-I genes (Chaves et al. 2009; Kaufman et al. 1999; Shiina et al. 2004; Wang et al. 2012). The size of the chicken TAP genes is also much smaller compared to their human orthologs (TAP1 being 61% the size of human TAP1 and TAP2 being 34% of size the human TAP2). In addition, the chicken TAP1 and TAP2 genes have an overall identity with human TAP1 and TAP2 of 36% and 49%, respectively. Interestingly, when comparing the TAP sequences from 11 different species, human included, the least conserved domain in the TAP genes was shown to be the tapasin-binding domain (Walker et al. 2005). As stated previously very little is known about the MHC organisation and gene localisation of the songbird MHC.
However, there are indications pointing to TAP genes mapping to a different chromosome than the MHC-I genes in the zebra finch that might suggest that this could also be the case for other songbirds (Balakrishnan et al. 2010).

As described in more detail above, human TAP proteins translocate a pool of peptides, that could potentially bind different MHC-I allomorphs, into the ER lumen (Obst et al. 1995). In chickens on the other hand the TAP genes have been shown to be more functionally polymorphic as compared to human TAP genes (Obst et al. 1995; Walker et al. 2005), which generates a functional diversity that results in a higher specificity in the peptide pool translocated into the ER lumen (Walker et al. 2011). In addition, the specificity of the TAP allomorphs seem to correspond to the specificity of the MHC-I allomorph that is dominantly expressed in a particular individual suggesting a linkage between and coevolution of the MHC-I and TAP genes promoting a shared peptide specificity between these proteins (Walker et al. 2011). Based on this shared specificity between MHC-I and TAP, coevolution has been proposed to be one reason that could explain the single dominantly expressed MHC-I molecules seen in these birds (Walker et al. 2011). Interestingly, in a recent study on zebra finches no polymorphism in the TAP genes could be observed (Ekblom et al. 2011), which might indicate that the songbird TAP proteins are more functionally similar to their human orthologs in regards to translocation of a broader spectrum of peptides as compared to the more specific peptide translocation seen in the chicken.
Tapasin

In humans, tapasin has been shown to have low polymorphism with no functional implications (Copeman et al. 1998; Furukawa et al. 1998; Herberg et al. 1998), whereas in the chicken, tapasin genes have been shown to be highly polymorphic (van Hateren et al. 2013). The functional importance of tapasin in the antigen presentation of the chicken has only recently been investigated and the findings from one study suggests that the presence or absence of tapasin affect different chicken MHC-I allomorphs differently in regards to initial binding and dissociation of peptides (van Hateren et al. 2013). These results are in line with what has been shown in human systems with different MHC-I allomorphs being differently dependent on tapasin for their cell surface expression (Peh et al. 1998). However in chicken, it has been shown using a cellular system that when a MHC-I allomorph from one tapasin-MHC-I haplotype was introduced together with a tapasin allomorph from another tapasin-MHC-I haplotype the maturation of the MHC-I molecule was impaired (van Hateren et al. 2013). This suggests that in chicken, different tapasin allomorphs exert different effects on specific MHC-I allomorphs. Based on these findings it has been suggested that coevolution could also have taken place between chicken MHC-I and chicken tapasin in a similar way as for TAP and MHC-I (van Hateren et al. 2013).
MHC-I and disease

MHC-I molecules present peptides generated from intracellular self-protein, pathogens and tumours to cytotoxic T cells. T cells start a cytolytic attack against cells recognised as either tumour cells or cells infected by an intracellular pathogen resulting in elimination through apoptosis. However, both tumours and several different viruses have developed immune evasion strategies that target the MHC-I antigen presentation pathway to avoid clearance by the cytotoxic T cells.

Tumour immune evasion strategies

Most cancer types exhibit between 30-80% impairment in MHC-I antigen presentation and this impairment is often seen already early on in malignant transformation (Garrido et al. 2010). The defects that result from the tumour evasion strategies can be divided into two groups, reversible and irreversible (Garrido et al. 2010). The reversible defects are controlled by repression of gene transcription through different oncogenes e.g., Her2/Neu and HPV E7 (Georgopoulos et al. 2000; Mimura et al. 2011). The activation of these oncogenes most often leads to histone modifications that decrease the gene expression of MHC-I and related proteins. However, such modification can be restored by DNA de-methylating agents and inhibitors of de-acetylases (Garrido et al. 2010; Khan et al. 2008; Rodriguez et al. 2007). In addition, as most genes involved in MHC-I antigen presentation encode a so-called interferon stimulated responsive element (ISRE) in their promoter regions, treatment with immunostimulatory cytokines such as interferons can also
restore MHC-I expression (Seliger et al. 2008). Proteins in the MHC-I quality control pathway such as tapasin and TAP have been shown to be commonly down-regulated in different tumours due to gene repression (Facoetti et al. 2005; Lopez-Albaitero et al. 2006; Seliger et al. 1996). In contrast to the reversible defects just described, the irreversible defects are most often a result from structural defects, such as different point mutations of the MHC-I, the β2m and other antigen presentation related genes (Lampen and van Hall 2011). Studies have shown that these structural defects are frequently found in some cancer types however, most MHC-I defects seen in tumours are suggested to be due to the reversible defects (Garrido et al. 2010; Koopman et al. 2000).

**Viral immune evasion strategies**

The goal of any virus is to be able to freely replicate in the infected cell without being recognised by the cytotoxic T cells. To achieve this goal several viruses have evolved strategies to evade and disrupt different parts of the MHC-I antigen presentation pathway in order to decrease the cell surface expression of MHC-I. Two important components of the PLC are TAP and tapasin and both these proteins have been shown to be targets for viral immune evasion strategies. I have here chosen to exemplify viral immune evasion by describing the evasion strategies of four common viruses, which mechanisms have been well described in the literature.
Strategies targeting TAP

The human herpes simplex virus (HSV) encodes a protein called infected cell protein 47 (IC47) that by binding with high affinity to the cytoplasmic domain of TAP inhibits peptide-binding and thus also ATP hydrolysis and peptide translocation (Ahn et al. 1996; Tomazin et al. 1996). Additionally, although in a manner different from that of the HSV protein IC47, the human cytomegalovirus (HCMV) also encodes a protein, US6, that targets the function of TAP. The US6 is an ER resident protein that acts by binding to the ER luminal domain of TAP resulting in a conformation change that inhibits ATP-binding, which results in inhibition of peptide translocation from the cytosol into the ER (Hewitt et al. 2001; Kyritsis et al. 2001). The Epstein-Barr virus (EBV) protein BNLF2a also blocks the function of TAP by arresting TAP in a conformation that is incapable of transporting peptides into the ER lumen (Wycisk et al. 2011).

Strategies targeting tapasin

The MHC-I dedicated protein tapasin is a key player in MHC-I quality control thus making it an excellent target for viral immune evasion strategies. The Adenovirus E3 is one of the viruses that targets tapasin in an indirect manner. The E3/19K protein encoded by the Adenovirus E3 is a transmembrane glycoprotein that binds to TAP and inhibits tapasin-binding (Bennett et al. 1999). It is not yet known exactly how the E3/19K protein inhibits the interaction between TAP and tapasin, but two possible strategies have been suggested; (I) the E3/19K binds to TAP and thereby induces a
conformational change that prevents tapasin from binding or, (II) binding to
the tapasin-binding site of TAP thus blocking this region for tapasin (Bennett
et al. 1999).

HCMV also targets the function of tapasin. The binding of the HCMV
protein US3 to tapasin inhibits its function by blocking tapasin dependent
peptide loading and optimisation of the peptide cargo of MHC-I molecules
(Park et al. 2004). HCMV has also been suggested to obstruct tapasin gene
transcription by interfering with the positive regulator domain 1 (Halenius et
al. 2011). This domain has been shown to bind to the tapasin promoter and
inhibit IFNγ-induced transcription of tapasin (Doody et al. 2007). The effects
of these different evasion strategies on the quantity of cell surface expressed
MHC-I molecules varies depending on the tapasin dependency of the MHC-I
allomorphs presented by the infected cell as described above. In addition to
affecting the quantity of MHC-I molecules the peptide repertoire might also
be affected and this can further affect the ability of cytotoxic T cells to
recognise the HCMV infected cells.

**HLA-I and disease association**

Within the human population over 5800 different allomorphs
(http://hla.alleles.org/nomenclature/stats.html) are known to date (January
2014) and this large MHC-I diversity is believed to be maintained as a
response to the pathogens present in our surroundings. Although, associations
between infectious disease and specific HLA-I allomorphs are very rare some
associations have been reported for specific HLA-I allomorphs and malaria, HIV/AIDS and hepatitis B (Gilbert et al. 1998; Goulder and Walker 2012; Hill et al. 1991; Lyke et al. 2011; Singh et al. 2007). In addition to HLA-I specific associations with infectious disease, the autoimmune disease AS has a well-documented association with the HLA-B*27:05 allomorph (Brewerton et al. 1973; Schlosstein et al. 1973). Also other HLA-I allomorphs of the B27 supertype has been shown to be associated with AS (Ramos and de Castro 2002).

**Ankylosing spondylitis**

AS is a form of arthritis that can cause severe chronic pain. It predominantly affects the spine and it causes inflammation of the spinal joints. In very severe cases of AS the inflammation can give rise to new bone formation on the spine, causing the spine to fuse in a fixed and immobile position (Braun and Sieper 2007; Sieper et al. 2002) The exact role of HLA-B*27:05 in the onset and/or progression of AS is still not known but there are several hypothesis on the subject. It has been proposed that the involvement of HLA-B*27:05 is due to its presentation of so-called arthritogenic peptides, pathogen derived peptides that mimic self-peptides, and when these peptides are presented by HLA-B*27:05 they would activate autoreactive T cells thereby generating an autoimmune response (Benjamin and Parham 1990). The accuracy of this hypothesis has been debated but so far it has not been either completely proven or disproven (Lopez de Castro 2007). In addition, it has been shown that tapasin affects the quality of the peptide repertoire presented by HLA-B*27:05 (Purcell et al. 2001; Sesma et al. 2005).
Based on this one could speculate that dysregulation of tapasin could play a role in the pathogenesis of AS.

The HLA-B^27:05 is a unique molecule in several ways. It has been suggested that its propensity to misfold and form aberrant conformations form the link to AS (Allen et al. 1999; Bowness 2002; Mear et al. 1999). The propensity of HLA-B^27:05 to misfold has been suggested to involve the reactive unpaired cysteine, Cys67, present in the α1 domain of this molecule (Dangoria et al. 2002; Weiss et al. 1996; Whelan and Archer 1993). Few other HLA-I molecules have been shown to share this feature of a reactive unpaired cysteine at position 67 making the HLA-B^27:05 rare in this aspect (Kostyu et al. 1997). The Cys67 has been shown to be critical in the formation of homodimers when HLA-B^27 HC are allowed to refold in the absence of β₂m (Allen et al. 1999) and point mutation exchanging the Cys67 results in prevention of homodimer formation (Allen et al. 1999; Dangoria et al. 2002). In addition to Cys67, the structural cysteine Cys164 has been suggested to be implicated in the formation of homodimers of HLA-B^27:05 (Antoniou et al. 2004). Antoniou and colleagues showed that a slower maturation rate increased the formation of homodimers of both HLA-B^27:05 and HLA-A^02:01 (Antoniou et al. 2004). Moreover, the presence of Cys67 in HLA-B^27:05 slows down the maturation rate of these HLA-I molecules in the ER (Antoniou et al. 2004).
Interestingly, even though HLA-B*27:05 has been shown to be rather tapasin independent in regards to stable surface expression (Peh et al. 1998), the absence of tapasin has been shown to increase the amounts of free HC within the ER of tapasin deficient cells (Montserrat et al. 2006). In addition it was shown that formation of heterodimeric forms of HLA-B*27:05 was more efficient in the presence of tapasin as compared to in its absence. One could speculate that this increase in free HC within the ER might lead to an increase in formation of aberrant conformations of HLA-B*27:05 such as homodimers.

Misfolding and formation of aberrant conformations can take place either within the ER lumen or as a result of endosomal recycling of cell surface expressed molecules (Bird et al. 2003; Dangoria et al. 2002). In the ER, the misfolding of HLA-B*27:05 during MHC-I maturation has been shown to lead to accumulation of misfolded protein that can trigger ER stress, thereby leading to activation of the unfolded protein response (UPR), which eventually can lead to apoptosis of the cell (Colbert 2000b; Dangoria et al. 2002; Schroder and Kaufman 2005). Cell surface expressed HLA-B*27:05 have been suggested to, during dissociation of the heterodimers and subsequent endosomal recycling, form aberrant conformations, such as homodimers that are re-expressed at the cell surface (Bird et al. 2003). These aberrant conformations of HLA-B*27:05 are not thought to be recognised by the TCR of the cytotoxic T cells but rather studies have shown that these conformations can be recognised by other leucocyte receptors, e.g., LILRA1, LILRB2, KIR3DL1 and KIR3DL2 that are present on both NK cell and T lymphocytes (Allen et al. 2001; Chan et al. 2005; Kollnberger et al. 2002).
Further studies need to be performed to understand the implications of misfolding and aberrant conformation of HLA-B*27:05 in the AS pathogenesis.

Polymorphism and variance in expression of the aminopeptidases ERAP1 and ERAP2 have in addition to HLA-B*27:05 been shown to be linked to AS (Campbell et al. 2011; Haroon and Inman 2010; Keidel et al. 2013; Tsui et al. 2010). Polymorphism in ERAP1 has been shown to significantly change the peptide pool available for MHC-I loading (García-Medel et al. 2012). Interestingly, it has been suggested that a decrease in ERAP1 activity results in protection against AS whereas an increase in ERAP1 activity results in an increased susceptibility to AS (Alvarez-Navarro and Lopez de Castro 2014; Campbell et al. 2011; García-Medel et al. 2012; Keidel et al. 2013). Taken together this suggests that the trimming of peptides that can later be presented by HLA-B*27:05 are of functional relevance in AS pathogenesis. One could speculate that an over expression of ERAP1 could diminish the HLA-B*27:05 binding peptide pool, which may result in a higher proportion of aggregated HLA-B*27:05 molecules in the ER leading to ER stress and activation of the UPR. In addition, significant changes in the peptide pool due to ERAP1 polymorphism may also affect HLA-B*27:05 stability, folding and homodimer formation at the cell surface (Marcilla and Lopez de Castro 2008).
**Immunotherapy and MHC-I antigen presentation**

The use of immunotherapy has been proven efficient in treatment of tumours of different kinds (Giannopoulos et al. 2003; Pizzitola et al. 2014; Secondino et al. 2013). To succeed with immunotherapy several things are of importance including disruption of immunosuppression and increasing the immunogenicity of the tumour cells.

In tumour cells different parts of the antigen presentation pathway, such as TAP and tapasin, can be dysregulated leading to a changed MHC-I presentation in turn resulting in alterations and decreased immunogenicity (Lopez-Albaitero et al. 2006; Seliger et al. 1996). For this reason it is of great value for therapeutic purposes to find ways to modulate and restore the MHC-I cell surface expression. Treatment with cytokines such as IFN-γ has been shown to increase expression of certain proteins within the MHC-I antigen presentation pathway resulting in an increased cell surface expression of MHC-I (Lopez-Albaitero et al. 2006; Ritz et al. 2001; Seliger et al. 1996). However, a high MHC-I cell surface expression does not necessarily mean activation of T cells, as this is also dependent on the peptides presented by the MHC-I. Thus, the use of different substances that merely boost the amount of MHC-I at the cell surface is alone not likely to be sufficient to increase the cells immunogenicity. Instead a combination of increased MHC-I expression and the addition of immunogenic peptides are preferred and would enable a specific T cell response, resulting in a specific clearance of tumour cells.
DISCUSSION OF THE ARTICLES

Paper I

In silico peptide-binding predictions of passerine MHC class I reveal similarities across distantly related species, suggesting convergence on the level of protein function

Background

The MHC-I genes are the most polymorphic genes known to date and the proteins that they encode play an important role in both the adaptive and the innate immune defence. In humans three different classical MHC-I genes are found, the HLA-A, -B and -C. Studies in other vertebrates such as birds have shown that the number of MHC-I genes varies greatly between different species e.g., in chicken only two classical MHC-I genes has been found whereof only one is dominantly expressed (Guillemot et al. 1988; Kaufman et al. 1999; Wallny et al. 2006). In contrast other birds, such as songbirds, have been shown to have a larger number of MHC-I genes, between four and eight, and in some songbirds as many as 16 different MHC-I gens have been reported (Bonneaud et al. 2004; Promerova et al. 2012; Schut et al. 2011; Sepil et al. 2012; Westerdahl et al. 1999; Wittzell et al. 1998). The variations in MHC-I between different species are intriguing to us and we wanted to further explore what the reasons behind them could be.
**Aim**

The aim of this study was to investigate the peptide-binding of MHC-I molecules in songbirds and to see if or how these different molecules are related on a functional level. We envision that our findings would result in a better understanding of the high number of MHC-I genes found in many songbirds. We studied MHC-I genes from three different passerine species; the great read warbler, the house sparrow and the tree sparrow.

**Methods**

In this paper we used different approaches to study songbird MHC-I genes, both from a genetic and a functional point of view. We started out by studying 14 MHC-I genes from a genetic perspective using sequence analysis to find sites under positive selection, that is sites where a change in amino acid is selected for during evolution as they contribute to a better fitness for the individual. In addition we characterised the genetic relationship between these MHC-I genes. Next we wanted to characterise the functional relationships of the resulting proteins from the 14 MHC-I alleles. We chose an *in silico* approach that used the predicted peptide-binding preferences of the 14 MHC-I molecules and clustered the molecules based on degree of similarity in regards to peptide-binding (Thomsen et al. 2013). All *in silico* methods for peptide-binding predictions available today are based on pMHC-I binding data from several different peptides and MHC-I allomorphs. To get the most accurate predictions for the passerine MHC-I molecules it was important to first find a prediction method that was good at predicting pMHC-I binding in
non-humans. We thus chose to start with a method called \textit{NetMHCpan} (Hoof et al. 2009; Nielsen et al. 2007) as it has been shown to have high performance accuracy as compared to other prediction methods (Zhang et al. 2012), and not least high performance accuracy for non-human MHC-I molecules (Hoof et al. 2009; Nene et al. 2012; Pedersen et al. 2011). During the work on this project it was discovered that when the MHC-I molecules studied had a very distant relationship to the MHC-I molecules in the training data of \textit{NetMHCpan}, a combination of prediction methods outperformed \textit{NetMHCpan} alone (Karosiene et al. 2012). Based on these studies and the fact that no peptide-binding data was available for any of the three species that we studied, we chose to use the \textit{NetMHCcons} method (Karosiene et al. 2012). \textit{NetMHCcons} is a consensus method of three previously described prediction methods, the \textit{NetMHC} (Lundegaard et al. 2008), the \textit{NetMHCpan} (Hoof et al. 2009; Nielsen et al. 2007) and the \textit{PickPocket} (Zhang et al. 2009). To illustrate the functional relationship between the 14 different MHC-I molecules a tree based on peptide-binding preferences was generated.

\textit{Results and discussion}

Human MHC-I allomorphs have previously been shown to be significantly different in their peptide-binding specificity (Rammensee et al. 1995). Here we showed that also the passerine MHC-I allomorphs differ in their peptide-binding preference.
The analysis of the genetic relationship between the 14 MHC-I alleles clearly showed two clusters separated with high bootstrap support among the sparrows. Interestingly, the two sparrow species were spread across the two clusters suggesting that the genetic difference that we see between the different MHC-I genes predates the separation of these two species. The great reed warbler MHC-I alleles also formed a separate cluster that was significantly separated from the sparrow clusters. In contrast to the tree based on genetic analysis, the tree based on functional analysis reveals an overlap between the great reed warbler allomorphs and two house sparrow allomorphs. This suggests that the MHC-I allomorphs from the house sparrow and the great reed warbler might have evolved under similar pathogen pressures resulting in an overlap in peptide-binding preference.

The data from *in silico* methods should always be interpreted with caution, as these are merely predictions of reality. The performance of an *in silico* method is based on the quality and quantity of the data that it has been trained with. Thus it is important to choose the *in silico* method that is most optimal for your application. The use of different prediction methods can be greatly beneficial e.g., in terms of finding potential T cell epitopes as a huge number of peptides can be checked for potential MHC-I binding in a very cost efficient way.
Conclusions

We found differences on the level of MHC-I alleles and allomorph characteristics rather than on a species level. Based on these findings we suggest that convergent evolution on the level of the protein function, potentially driven by selection from shared pathogens, has resulted in allomorphs with similar peptide-binding repertoires. In addition we also conclude that the high number of MHC-I genes found in these three passerines are of functional importance based on the differences that they exhibit in peptide-binding preference.

Paper II

Great reed warbler major histocompatibility complex class I molecules with considerable differences on the level of amino acids share peptide preferences

Background

In paper I we investigated the function of MHC-I molecules in songbirds in regards to peptide-binding specificities: 14 different MHC-I allomorphs from three different species were studied using in silico peptide-binding prediction methods. We found differences on the level of MHC-I allomorph characteristics rather than on a species level and we also concluded that the large number of MHC-I genes found in songbirds are of functional importance (Follin et al. 2013).
Aim

To further investigate the functional relevance of a high number of MHC-I genes we here chose two great reed warbler allomorphs, with a high number of differences in the primary sequence, to study function in more detail using recombinant proteins and a biochemical approach. The specific aims of this study were to investigate the MHC-I complex formation and stability of two great reed warbler MHC-I allomorphs using a biochemical approach and then compare these results with the previous in silico peptide-binding predictions from paper I.

Methods

We chose two great reed warbler allomorphs, Acar_5 and Acar_19, which have been previously described and characterised (Follin et al. 2013; Westerdahl et al. 1999). In addition we included the well-characterised human HLA-I allomorph HLA-B*08:01 for comparison. The Acar_5 and Acar_19 allomorphs were chosen for further studies based on the high number of differences between these allomorphs in the primary sequence (Westerdahl et al. 1999). We produced recombinant Acar_5, Acar_19 and HLA-B*08:01 HC and recombinant human and great reed warbler β2m proteins as previously described (Pedersen et al. 2001).

To study the MHC-I complex formation and stability of these two great reed warbler allomorphs we used a scintillation proximity assay (SPA) based
method to study pMHC-I dissociation as described previously (Harndahl et al. 2011). Briefly, we used $^{125}$I labelled $\beta_2$m mixed with biotinylated HC and peptide in a streptavidin coated scintillation plate. The peptide-HC-$\beta_2$m mix was then left to fold and form complexes. The proximity of the $^{125}$I to the plate generates a light emission that can be measured to quantify the amount of complexes. The light emission is then measured at several time points to obtain a dissociation curve from which the specific half-life of the peptide-allomorph complex can be calculated.

Using $X_n$-libraries, libraries with random peptides of between 7 and 13 amino acids in length, the MHC-I complex formation and stability assay was used to determine the length preference of the two great reed warbler allomorphs. Next we determined the peptide-binding motifs by using a positional scanning combinatorial peptide library (PSCPL) consisting of 180 sub-libraries and one reference library in combination with the SPA. Based on the two unique peptide-binding motifs that we generated for the Acar allomorphs we selected 184 peptides for each allomorph that best corresponded to their unique peptide-binding motifs. These peptides were then used to study pMHC-I complex formation and stability.

To improve on the \textit{in silico} peptide-binding prediction methods that we used in paper I we used the new functional pMHC-I stability data obtained in this study to retrain the old version of NetMHCcons (Karosiene et al. 2012).
Results and discussion

Human MHC-I molecules have previously been shown to prefer peptide lengths of 8-10 amino acids (Rammensee et al. 1995). Here we show that the two great reed warbler allomorphs also bind 8-10-mers but not significantly more than they bind 11-13-mers. In addition we show that both allomorphs form significantly less complexes with the shorter 7-mer peptides as compared to 9-11-mers. The complexes formed with 7-mers also showed a significantly lower complex stability as compared to 9-mers for both allomorphs. The most stable complexes were formed with 9-mers although these were not significantly more stable as compared to 8, 10, 11 and 12-mers. These results are in line with previous results from our research group were we have shown that the human HLA-A*02:01 molecule forms complexes of unsuspected high stability with peptides longer than the traditionally considered optimal lengths of 8-10 amino acids (Geironson et al. 2013; Rammensee et al. 1995).

In paper I we showed using an in silico approach that both the Acar_5 and the Acar_19 allomorphs have three anchor positions, position 1, 2 and 9 of the peptide (Follin et al. 2013). By using the PSCPL libraries we could determine the exact peptide-binding motifs of these two molecules. The peptide-binding motifs showed the same three anchor position but with addition of two additional positions important for peptide-binding, positions 3 and 5. This is interesting not least since position 5 is seldom seen to influence peptide-binding to human HLA-I molecules (Lund et al. 2004).
However, our previous results using *in silico* methods also indicated that position 5 could be important for peptide-binding, although not for great reed warblers but for other songbirds (Follin et al. 2013).

By using the two groups of distinct peptides (184 peptides per allomorph) that were selected for each great reed warbler allomorph we could generate pMHC-I stability data for *Acar_5* and *Acar_19*. We found that the peptide pools that could bind to these great reed warbler allomorphs and form stable complexes were overlapping to a high degree, which suggests similar peptide-binding preferences. This is in spite of the high polymorphism observed for these allomorphs on a genetic level.

We then chose to compare the two great reed warbler allomorphs with the human allomorph HLA-B*08:01 in regards to pMHC-I complex stability. Here we show that the kinetics of pMHC-I dissociation for these bird molecules are similar to complex dissociation kinetics seen for the human molecule, HLA-B*08:01. This suggests that the complexity seen in the dissociation for these molecules is a result of the presence of pMHC-I complexes of several different maturation stages. This is in line with what is known about the MHC-I maturation in the ER where other proteins, such as tapasin, are of importance for proper maturation.
Based on the functional pMHC-I complex stability data, we were able to retrain the NetMHCcons and NetMHCcluster methods that we used in the in silico analyses in paper I. Then we generated a new tree, closer to reality, based on peptide-binding specificity. This new tree showed a partly different pattern as compared to the tree shown in paper I (Follin et al. 2013). The most interesting difference observed was that the cluster with two house sparrow allomorphs and one great reed warbler allomorph (Acar_3) disappeared and interestingly Acar_3 moved and clustered with the sparrow allomorphs. Acar_3 seems to share binding preferences with sparrow allomorphs, a finding that was consistent between trees. In addition, in the new tree we observed a closer clustering of the other five great reed warbler allomorphs.

We have used a biochemical approach with recombinant proteins to study the interactions between peptide, MHC-I HC and β2m from the great reed warbler, proteins that have never been studied in regards to their functional properties before. The advantage of using recombinant proteins and biochemical assays, over cellular systems or in vivo models, is that the system in many ways can be more easily controlled which allows more direct studies of specific molecular interactions. Using recombinant proteins may be of particular advantage when the study animal is wild, and difficult to keep in captivity, and no established cell lines exist. The biochemical assays are a great asset to understand protein-protein interactions although it is important to note that results obtained from biochemical assays should only be extrapolated with caution to in vivo systems.
Conclusions

We found that the two great reed warbler allomorphs had a similar length preference for peptides as human HLA allomorphs. In addition, we showed that even though the two MHC-I genes were highly polymorphic on a sequence level the peptide-binding preference was overlapping. In addition, the complexity of the pMHC-I dissociation, similar to the HLA-B*08:01, imply that these songbird molecules are dependent on a stepwise maturation pathway that involves other proteins such as tapasin. Finally, the incorporation of novel pMHC-I complex stability data into the in silico prediction methods changed the outcome of the predictions compared to previous analyses. This further strengthens the notion that the more data from different species that is incorporated the better the prediction method will perform.
Paper III

Stability and conformation of HLA-I allomorphs are influenced by tapasin

Background

Tapasin is a multi functional protein that plays an important role in the HLA-I quality control by optimising the peptide cargo of HLA-I molecules (Paulsson et al. 2006; Paulsson and Wang 2004; Roder et al. 2011). Studies have shown that the dependence on tapasin in HLA-I maturation differs greatly between different allomorphs (Barber et al. 2001; Geironson et al. 2013; Peh et al. 1998). In addition, in the absence of tapasin both the quantity and quality of the presented peptide-HLA-I (pHLA-I) complexes are altered (Peh et al. 1998; Zarling et al. 2003).

Studies have shown that certain HLA-I alleles are associated with disease e.g., HLA-B*27:05 and AS (Brewerton et al. 1973; Schlosstein et al. 1973). The exact mechanisms behind HLA-B*27:05 involvement in AS is not yet known however different theories have been proposed (Lopez de Castro 2007). One theory is that HLA-B*27:05 misfold and form aberrant conformations, which increase the tendency of HLA-B*27:05 to aggregate during early maturation (Allen et al. 1999; Bowness 2002; Kollnberger et al. 2004; Mear et al. 1999). The aggregate formation results in protein accumulation in the ER, which leads to the onset of the UPR suggested to be involved in the autoinflammatory reaction of AS (Colbert 2000a).
Recent studies have shown that polymorphism and variance in expression levels of the aminopeptidases ERAP1 and ERAP2 might also be linked to AS (Campbell et al. 2011; Haroon and Inman 2010; Keidel et al. 2013; Tsui et al. 2010).

**Aim**

Our group and others have shown that HLA-B*27:05 is dependent on tapasin to only a low degree for complex formation and cell surface expression (Geironson et al. 2013; Peh et al. 1998). This made us want to further investigate the implication of tapasin and the quality control pathway in misfolding and formation of aberrant conformation of HLA-B*27:05 and two other HLA-I allomorphs. The aim of this study was specifically to investigate the role of tapasin in surface stability of three different human HLA-I allomorphs, HLA-B*08:01, HLA-B*27:05 and HLA-A*02:01. We also wanted to investigate how tapasin influences the formation of aberrant HLA-I conformations and their expression and stability at the cell surface.

**Methods**

In this study we chose to use a cellular system to investigate the role that tapasin plays for pHLA-I complex stability. We used cells that were either proficient or deficient in tapasin and that were transfected with one of the three allomorphs HLA-B*08:01, HLA-B*27:05 or HLA-A*02:01. We started with analysing the stability of surface expressed pHLA-I in the presence and
absence of tapasin. This was done by treating cells with brefeldin A (BFA), to block transport of newly synthesised HLA-I molecules out to the cell surface, and then analysing the cell surface expression of W6/32 reactive epitopes at different time points using flow cytometry. The W6/32 antibody recognises HLA-I HC associated with peptide and β2m and to some extent also HLA-I homodimers (Allen et al. 1999; Parham et al. 1979).

Next we investigated the presence of aberrant conformations of the different allomorphs. This was first done using western blot performed under non-reducing conditions with lysates from tapasin deficient or proficient cells transfected with HLA-B*08:01, -B*27:05 or -A*02:01. To study the cell surface expression of aberrant HLA-I conformations we stained the cells with HD6, which has been shown to recognise non-conventional HLA-B*27:05 conformations (Payeli et al. 2012), and HC10, which has been shown to recognise β2m free HCs of most HLA-B and -C allomorphs and some HLA-A allomorphs and performed flow cytometry analysis (Stam et al. 1986) In addition, we studied the stability of HD6 and HC10 reactive MHC-I conformations in the presence and absence of tapasin and hence the cells were treated with BFA at different time points. Next we wanted to further investigate the stability of the non-conventional forms i.e., homodimers found in HLA-B*27:05 cells proficient or deficient in tapasin. This was done using mild acid treatment and cells were stained for cell surface expression of W6/32, HD6 and HC10 reactive epitopes and analysed with flow cytometry.
To study the peptide length preference and complex stability of the HLA-B*27:05 allomorph, we used a biochemical assay, the SPA described previously (Harndahl et al. 2011), in combination with Xn-peptide libraries, containing peptides of 7-13 amino acids in length.

Results and discussion

It has previously been shown that different HLA-I allomorphs differ in their dependence on tapasin (Geironson et al. 2013; Peh et al. 1998). We here showed that out of the three allomorphs studied, the HLA-B*08:01 and the HLA-A*02:01 are positively affected by the presence of tapasin in regards to cell surface stability. Interestingly, we could also see that the HLA-B*27:05 was not more stable in the presence of tapasin, rather a slightly faster dissociation could be observed for this allomorph. Based on findings that non-conventional forms of HLA-B*27:05 can be recognised by the W6/32 antibody (Allen et al. 1999) one could speculate that the higher stability of W6/32 reactive epitopes of HLA-B*27:05 in tapasin deficient cells may be a result of formation of more non-conventional epitopes in the absence of tapasin.

HLA-B*27:05 has been shown to form aberrant conformations and to further elucidate this we did western blot analysis with an antibody against MHC-I HC under non-reducing conditions. We found that in lysates from HLA-B*27:05 both in tapasin deficient and proficient cells, a higher molecular weight species was present that in size corresponded to homodimers.
The presence of HLA-B*27:05 homodimers was further supported by the finding of HD6 reactive epitopes at the cell surface of cells expressing HLA-B*27:05. Interestingly, the expression of HD6 reactive epitopes is higher on tapasin deficient cells as compared to on tapasin proficient cells. In addition, we show that the HC10 reactive epitopes are found to a higher degree on tapasin deficient HLA-B*27:05 cells as compared to the tapasin proficient cells. It has been shown that the maturation of HLA-B*27:05 in the ER is more efficient in the presence of tapasin and that slow maturation of HLA-B*27:05 might actually increase the formation of aberrant conformations (Antoniou et al. 2004; Montserrat et al. 2006). Taken together with our results, this suggests that tapasin does play a role in decreasing the amount of cell surface expressed aberrant conformations of HLA-B*27:05. In addition, we saw that when HLA-B*27:05 cells were treated with mild acid buffer the amount of HC10 reactive epitopes increased on both tapasin proficient and tapasin deficient cells. We also saw that the amount of HD6 reactive epitopes was increased on tapasin proficient but not on tapasin deficient cells after mild acid treatment. One could speculate that the reason behind this is that when the heterodimeric molecules on the tapasin proficient cells are treated with mild acid they fall apart in a stepwise manner that reveals an HD6 reactive epitope. In contrast, the constant amount of HD6 reactive epitopes found on tapasin deficient cells even after mild acid treatment could be explained by an initial high amount of aberrant conformations at the cell surface and a one-step dissociation pattern of these molecules.
In connection to the expression of HLA-B*27:05, polymorphism and variance in expression of ERAP1 and ERAP2 has been shown to be linked to AS (Campbell et al. 2011; Haroon and Inman 2010; Keidel et al. 2013; Tsui et al. 2010). For this reason we chose to further investigate the influence that peptide length had on HLA-B*27:05 complex formation and stability. We here show that HLA-B*27:05 formed the most complexes with peptides of 10 and 11 amino acids in length, which is slightly longer than what is traditionally believed to be the optimal length for most MHC-I molecules (Rammensee et al. 1995). In addition, we show that the pHLA-I complexes formed with peptides of 7 amino acids in length were less stable as compared to complexes formed with longer peptides. These results suggest that HLA-B*27:05 prefers longer peptides and we speculate that an overexpression of ERAP leads to an increased peptide trimming and may result in interference with an optimal peptide loading that in turn can influence the maturation of HLA-B*27:05.

Conclusions

We found that tapasin has a positive effect on cell surface stability of W6/32 reactive epitopes for HLA-B*08:01 and HLA-A*02:01 but not for HLA-B*27:05. We also found that HLA-B*27:05 formed aberrant conformations both in the presence and absence of tapasin. However, the amount of cell surface expressed aberrant conformations was significantly higher in tapasin deficient cells which suggest that tapasin does play an important role in preventing presentation of aberrant conformations of HLA-B*27:05.
Paper IV

Modulation of HLA class I on the surface of tapasin deficient cells

Background

The maturation of HLA-I molecules occurs in the ER and it involves several different proteins that together constitute the antigen processing machinery (APM) (Neefjes et al. 2011). One such protein is tapasin, which plays an important role in shaping the peptide repertoire presented by HLA-I at the cell surface (Howarth et al. 2004; Purcell et al. 2001; Thirdborough et al. 2008). Exactly what the criteria are that defines which peptides that are eventually presented by HLA-I is not yet known. However, studies show that peptides that bind with high affinity to HLA-I and/or that can induce a stable pHLA-I complex make up the majority of surface expressed peptides (Howarth et al. 2004). Both viruses and tumours make use of evasion strategies that targets and down-regulates the expression of tapasin and other APM components in an attempt to inhibit optimal maturation of HLA-I resulting in suboptimal presentation of HLA-I at the cell surface (Roder et al. 2008; Seliger 2008). These changes most likely modify previously established immuno-dominance hierarchies leading to decreased T cell recognition.
**Aim**

The aim of this study was to investigate the cell surface stability of HLA-I molecules expressed in the absence of tapasin. In addition we wanted to explore the possibility to modulate the expression of HLA-I on tapasin deficient cells by addition of exogenous peptides or by addition of recombinant tapasin.

**Methods**

We used a cellular system to investigate the influence of exogenous addition of peptides and recombinant tapasin. Cells were first starved over night to increase cell surface expression of HLA-I molecules. They were then pulsed with either peptide or recombinant tapasin for 1h in the presence of BFA, added to block transport of newly synthesised HLA-I molecules out to the cell surface. After removal of peptide and recombinant proteins, cells were treated with BFA for different time points before analysis of their HLA-I cell surface expression. The conformation specific antibody W6/32 was used to stain cell surface expressed HLA-I molecules and the expression was then analysed using flow cytometry (Parham et al. 1979).
Results and discussion

We found that one out of three tested peptides were able to stabilise the cell surface expression of HLA-A*02:01 in a tapasin deficient cell line. After 4h of BFA treatment this stabilising effect was pronounced and significantly higher as compared to the other two tested peptides. Interestingly, when the same three peptides were exogenously added to a tapasin proficient cell line there was no change in stability for any of the peptides after 4h of BFA treatment. This suggest that cell surface expressed HLA-I molecules on tapasin deficient cells are loaded with peptides that has not induced a completely mature and locked conformation making it possible to increase stability of these molecules by adding optimal peptide.

To further investigate the possibility to stabilise the HLA-I cell surface expression on tapasin deficient cells by adding peptides exogenously, we tested a large set of peptides, predicted to bind with high affinity to HLA-A*02:01. Interestingly, we found that most of the tested peptides could stabilise the surface expressed HLA-I molecules initially. However, only a handful could increase stability over time. This suggests that affinity is not the only determining factor for HLA-I peptide-binding and formation of stable complexes. In support of this, when affinity and stability were compared for ability to start an immune reaction i.e., level of immunogenicity, stability was shown to be the superior predictor of immunogenicity (Harndahl et al. 2012).
Using recombinant proteins our group has previously revealed that a N-terminal part of tapasin (Tpn1-87) can promote formation of pHLA-I complexes (Roder et al. 2009; Roder et al. 2011). Here we showed that the highly tapasin dependent HLA-B*08:01 (Geironson et al. 2013) is stabilised by the exogenous addition of recombinant Tpn1-87. However, this effect is lost after 4h of treatment with BFA indicating that tapasin is not able to induce a more long-lasting stable conformation. The less tapasin dependent HLA-A*02:01 was not affected at all by the addition of exogenous recombinant Tpn1-87.

Conclusions

We found that addition of exogenous peptides could to some extent modulate the cell surface expression of HLA-A*02:01 in regards to stability. However, the selection of which peptides that can replace and induce stable conformations of HLA-I on the cell surface is still complex and further studies needs to be performed to resolve these criteria.
Vårt immunförsvar har till uppgift att skydda oss från olika former av virus och bakterier som kan orsaka infektioner och sjukdomar men också sjukdomar som uppstår till följd av cellförändringar, som till exempel cancer. I vårt immunförsvar finns många viktiga celler och molekyler. En sådan molekyl är MHC klass I som finns på ytan av alla celler som har en cellkärna. Dess uppgift är att visa hur cellen mår för andra celler som ingår i vårt immunförsvar. MHC klass I molekylen gör detta genom att binda in små bitar av protein (peptider) i vad som kallas för en bindningsklyfta för att sedan ta sig ut till cellytan för att visa upp dessa peptider. I cellen produceras mängder av olika peptider och om cellen är frisk så kommer endast kroppsegna peptider att produceras och presenteras av MHC klass I. Om cellen däremot är sjuk på något sätt, infekterad av virus eller om cellen blivit en cancercell så kommer MHC klass I att presentera peptider genererade från virusprotein eller tumörprotein för att visa att cellen inte mår bra. I vårt immunförsvar så har vi också specifika immunceller av olika slag som patrullerar runt i kroppen för att se till att alla celler i kroppen mår bra. En sådan celltyp är CD8+ T celler som har till uppgift att läsa av MHC klass I molekyler. När en MHC klass I molekyl presenterar en peptid som är av främmande ursprung, peptid från virus eller tumör, så kommer CD8+ T cellen att binda in till MHC klass I molekylen och märka detta och ta bort den sjuka och infekterade cellen.
En MHC klass I molekyl består av tre olika delar, en mycket variabel tung kedja, en liten invariabel kedja och en peptid. För att en MHC klass I molekyl ska presenteras bra på cellytan så är det väldigt viktigt att den peptid som den binder in i sin bindningsklyfta kan framkalla en viss stabilitet som gör att MHC klass I molekylen kan stanna kvar på cellytan tillräckligt länge för att bli upptäckt av CD8+ T cellerna. I vår kropp har vi mellan tre till sex olika varianter av MHC klass I molekyler och dessa olika varianter har olika preferenser för vilka peptider de vill binda i sin bindningsklyfta för vidare presentation på cellytan. Det är bara ett fåtal av de peptider som finns inuti cellen som har förmågan att binda och stabilisera MHC klass I molekylen därför behöver MHC klass I hjälp att hitta och binda in dessa peptider. En av dessa medhjälpare heter tapasin och man tror att tapasin hjälper till att stabilisera MHC-klass I molekylen så att flera olika peptider kan testas innan rätt peptid binder in. När rätt peptid har bundit in så känner tapasin av det och släpper iväg MHC klass I ut till cellytan. Ibland händer det att tapasin inte finns eller inte fungerar som det ska i vissa celler, vilket resulterar i att cellen presenterar färre MHC klass I molekyler på sin yta. Dessutom så binder då MHC klass I molekylerna in peptider med sämre förmåga att stabilisera molekylen vilket gör att de MHC klass I molekyler som presenteras på cellytan faller sönder snabbare och därmed hinner inte CD8+ T cellerna upptäcka de främmande peptiderna vilket i förlängningen leder till att sjuka celler inte tas bort.
I den här avhandlingen så har jag tittat närmare på hur de peptider som MHC klass I molekyler binder påverkar deras stabilitet och också vilken roll tapasin spelar i bildandet av stabila MHC klass I molekyler. I det första delarbetet (I) så valde vi att studera MHC klass I hos tre olika arter av småfåglar då det visat sig att dessa fåglar har ett högt antal olika MHC klass I gener jämfört med både andra fåglar och oss människor. Vi ville ta reda på om dessa olika gener gav upphov till MHC klass I molekyler med olika funktion. Detta gjorde vi genom att använda oss av en form av datorsimuleringar för att förutsöva vilka typer av peptider som skulle binda till vilka MHC klass I molekyler. Vi fann att när vi grupperade 14 olika MHC klass I molekyler baserat på vilka peptider de föredrog att binda till så bildades grupper med molekyler baserat på deras funktion snarare än vilken art molekylen tillhörde. Vi tror att detta beror på att dessa tre olika arter till viss del behöver kunna försvara sig mot liknande typer av sjukdomar och därför behöver deras MHC klass I molekyler kunna binda liknande peptider.

Som en uppföljning till delarbete (I) valde vi i delarbete (II) att tillverka två av de MHC klass I molekyler vi studerat i delarbete (I) för att kunna titta närmare på hur dessa molekyler bildas och vilka peptider de binder i verkligheten samt hur detta påverkar deras stabilitet. Vi fann att trots att vi valt två molekyler som var tämligen olika så föredrog de att binda till ungefär samma peptider. Vi fann också att stabiliteten och sönderfallet av dessa MHC klass I molekyler var väldigt likt vad vi känner till för MHC klass I molekyler hos människor. Detta tyder på att MHC klass I molekyler i småfåglar och människor fungerar på liknande sätt.
Vi valde sedan att använda de nya resultaten för vilka peptider de två MHC klass I molekylerna föredrog att binda för att ytterligare förbättra våra datorsimuleringar. Det visade sig att detta förändrade hur MHC klass I molekylerna från de tre arterna av småfåglar som vi studerade i delarbete (I) grupperade sig. Dock kvarstod att molekylerna bildade grupper baserat på funktion snarare än vilken art de tillhörde.

Man vet sedan tidigare att olika varianter av MHC klass I i människor är olika beroende av medhjälparen tapasin för att de ska kunna bli presentade på ett bra sätt på cellytan. I delarbete (III) så valde vi att titta närmare på hur tapasin påverkade presentationen av tre olika humana MHC klass I varianter. En av dessa varianter har dessutom visats vara associerad med utvecklandet av sjukdomen ankolyserande spondylit (AS). Vi fann att två av de tre studerade varianterna av MHC klass I presenterade färre molekyler på cellytan när tapasin inte fanns där. Dessa molekyler var dessutom mer instabila vilket tyder på att de inte lyckats binda in de bästa peptiderna utan hjälp av tapasin. Den tredje varianten av MHC klass I som också var den sjukdomsassocierade verkade inte vara särskilt påverkad av frånvaron av tapasin när det gällde antalet molekyler på cellytan eller deras stabilitet. Andra har tidigare visat att denna variant av MHC klass I har en förmåga att bilda avvikande former av MHC klass I där till exempel två tunga kedjor binder till varandra utan den lättta kedjan. Man tror att dessa former av molekylerna kan vara en del i sjukdomen AS. När vi studerade denna variant av MHC klass I i mer detalj så upptäckte vi att dessa avvikande former bildades i högre utsträckning när tapasin var frånvarande.
Detta tyder på att tapasin är viktigt för att förhindra bildandet av avvikande former av MHC klass I och kanske också för att förhindra utvecklandet av AS.

Sjukdomar så som cancer har en förmåga att störa funktionen av medhjälparen tapasin vilket som beskrivits ovan, kan leda till en försämrad presentation av MHC klass I molekyler på cellytan som i förlängningen leder till att kroppens immunförsvar inte kan bekämpa cancern på ett effektivt sätt. Ett sätt att försöka förbättra en sämre presentation av MHC klass I på cellytan är att sätta till peptider som kan förbättra molekylernas stabilitet och därmed öka chansen att cellen blir upptäckt av CD8+ T celler. I delarbete (IV) studerade vi hur vi med hjälp av olika peptider kunde förbättra uttrycket av MHC klass I på ytan av celler som saknande medhjälparen tapasin. Vi valde ut peptider som alla hade visat sig kunna binda till MHC klass I. Av alla peptider som vi testade så var det bara ett fåtal som faktiskt kunde binda in och stabilisera MHC klass I molekylerna som fanns på ytan av de celler som saknande tapasin. Vi valde att också testa samma peptider på MHC klass I som presentades på vanliga celler (celler som hade tapasin) och intressant nog så kunde inga av de testade peptiderna binda in och stabilisera MHC klass I på dessa celler. Vi tror att detta beror på att när medhjälparen tapasin finns i cellen vilket gör att MHC klass I kan binda in de allra bästa peptiderna redan inuti cellen och så behöver inga peptider bytas ut vid cellytan. När tapasin däremot inte finns så klarar MHC klass I inte av att själv hitta de bästa peptiderna utan presenterar istället peptider som bara är halvbra vilket gör det möjligt att byta ut vissa av dessa på cellytan genom att tillsätta bättre peptider.
Exakt vad som gör att en peptid binder bra och stabiliserar MHC klass I vet man ännu inte och fler studier behöver göras för att ta reda på detta.

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