

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Relevance of HLA Expression Variants in Stem Cell Transplantation

Britta Eiz-Vesper and Rainer Blasczyk  
*Institute for Transfusion Medicine, Hannover Medical School, Hannover  
Germany*

## 1. Introduction

Matching the donor and recipient for class I and II human leukocyte antigens (HLA) is pivotal to the success of allogeneic hematopoietic stem cell transplantation (HSCT). Transplantation across HLA barriers will lead to the development of T-cell responses to the mismatched HLA molecules, resulting in T-cell-mediated graft-versus-host disease (GvHD) or graft rejection in patients with insufficient immune suppression. The accuracy of testing and matching criteria has an important impact on the transplant outcome, but exact matching across multiple HLA loci (e.g., HLA-A, HLA-B, HLA-C, and HLA-DRB1) is a challenging task. Today, serological HLA diagnostic tests are being replaced by DNA-based typing methods considering only selected regions of the genes. Therefore, HLA null alleles or expression variants bearing their variation outside of these regions may be misdiagnosed as normally expressed variants, resulting in HLA mismatches that are highly likely to stimulate allogeneic T cells and trigger GvHD. This chapter will address the relevance, genetics, prevalence and diagnosis of HLA expression, variants of HLA class I loci and will discuss their clinical implications for transplantation.

## 2. The human major histocompatibility complex

The human major histocompatibility complex (MHC), also referred to as the human leukocyte antigen (HLA) complex, is encoded on the short arm of chromosome 6 (6p21) and is extremely polymorphic (Parham et al. 1988). HLA class I molecules are expressed on most nucleated cells. The HLA class I region comprises the gene loci for the heavy chains of the three classical human leukocyte antigens, HLA-A, -B, and -C. They consist of a heavy chain (44 kDa) and a non-covalently bound  $\beta$ 2 microglobulin ( $\beta$ 2m) light chain (12 kDa) encoded by chromosome 15. The heavy chain is made up of three extracellular domains:  $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 3. The highly polymorphic region of HLA class I molecules is located in the DNA and amino acid sequences of the  $\alpha$ 1 and  $\alpha$ 2 domains, which form the peptide-binding groove. Endogenous 8 to 12 amino acid peptides are presented to CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) (Natarajan et al. 1999). The  $\alpha$ 3 domain is mainly invariant and contains the binding site for the co-receptor CD8. Because of the MHC's role in recognizing pathogenic and cancerous peptides, these genes are under high environmental pressure to be very polymorphic. A total of 4,946 HLA class I alleles have been identified to date (<http://www.ebi.ac.uk/imgt/hla>; released April 2011).

HLA class I molecules are stabilized by disulfide bonds located in the  $\alpha 2$  and  $\alpha 3$  domains between cysteine (C) residues at amino acid positions 101/164 and 203/259. These bonds are essential for the correct processing and function of the molecules (Solheim 1999). Amino acid substitutions in these crucial C residues are likely to cause aberrant expression of the respective HLA class I molecules and may also change the affinity of the peptide-binding groove towards endogenous peptides (Warburton et al. 1994; Hirv et al. 2006; Hinrichs et al. 2009; Hinrichs et al. 2010).

HLA class II molecules (DR, DQ, DP) are mainly expressed on hematopoietic cells (macrophages, dendritic cells, T cells and B cells). The heterodimers are formed by two membrane-bound chains ( $\alpha$  and  $\beta$ ), each consisting of two domains ( $\alpha 1/\alpha 2$  or  $\beta 1/\beta 2$ , respectively) encoded by two genes co-located in the centromeric part of the MHC. The antigenic peptide (up to 30 amino acids) is presented to CD4<sup>+</sup> T helper cells (T<sub>h</sub> cells) in a cleft formed by the outermost  $\alpha 1$  and  $\beta 1$  domains. Nearly all of the polymorphisms occur at exon 2 of the respective A or B genes. Peptides presented by HLA class II molecules are derived from exogenous proteins as well as from epitopes of plasma membranes or endosomes (Rudensky et al. 1991; Chicz et al. 1993; Sant 1994). The nonpolymorphic  $\beta 2$  domain contains the binding site for the T cell co-receptor CD4. More than 1,457 HLA class II alleles have been identified to date (<http://www.ebi.ac.uk/imgt/hla>; released April 2011).

## 2.1 Peptide presentation by HLA

The ability to recognize and distinguish between self and non-self is primarily mediated by T lymphocytes, which survey the protein environment of cell surfaces for binding partners, i.e. for signs of foreign invasion. T cells do not recognize proteins directly; instead, they recognize imprints of ongoing protein metabolism in the form of peptides presented by HLA molecules. This phenomenon is called MHC restriction. The biological function of HLA molecules is to present antigenic peptides to T cells. Therefore, HLA molecules play a central role in T cell-mediated adoptive immunity. MHC class I molecules present peptides from endogenously synthesized proteins, whereas MHC class II molecules present peptides from incorporated exogenous proteins. All of these peptides originate from foreign or host cell proteins and are generated by proteasomal cleavage (class I pathway) or lysosomal processing (class II pathway). It has been estimated that about 0.5% of presented peptides are bound to MHC molecules, whereas more than 99% are ignored. Consequently, peptide binding to HLA is the single most selective event involved in antigen processing and presentation (Yewdell, Norbury, and Bennink 1999; Yewdell and Bennink 2001). A T cell-mediated immune response occurs when the T-cell receptor recognizes a specific peptide-MHC complex and thus identifies cells that have been infected by intracellular parasites or viruses or cells containing abnormal proteins (e.g., tumor cells). The peptides being part of a certain peptide-MHC complex triggering T-cell recognition are important tools for diagnosis and treatment of infectious, autoimmune, allergic and neoplastic diseases (Ferrari et al. 2000; Haselden, Kay, and Larche 2000; Singh 2000; Wang, Phan, and Marincola 2001). Different polymorphic HLA molecules have different peptide binding specificities (Falk et al. 1991; Sette et al. 1994; Bade-Doeding et al. 2007; Bade-Doeding et al. 2011). Peptides presented by MHC class I molecules are derived from cytoplasmic proteins by proteolytic degradation in the proteasome. Therefore, the MHC class I presentation pathway is often called the cytosolic or endogenous pathway. The MHC class I crystal structure features a

unique peptide-binding groove at the outer polymorphic  $\alpha 2$  and  $\alpha 3$  domains (Bjorkman et al. 1987, Madden et al. 1991). This groove can be subdivided into six pockets (A-F) of different size, shape, and function (Garrett et al. 1989; Matsumura et al. 1992). A pocket is defined as a unit having an affinity for a certain peptide side chain (e.g., affinity of pocket A for peptide position P1 and pocket B for P2). Some pockets have a well-shaped structure with an affinity for only one side chain, whereas others have an affinity for a group of side chains. In some cases, the boundaries between pockets are unclear. The most important residues and positions of a peptide are known as anchor residues and anchor positions. The identity and spacing of these primary anchors constitutes the peptide motif of an HLA specificity (Sette et al. 1987; Sette et al. 1989; Jardetzky et al. 1991; Ruppert et al. 1994; Rammensee, Friede, and Stevanoviic 1995). A typical peptide is 8 to 12 amino acids in length and binds in the peptide-binding groove, exhibiting an extended conformation with its terminal amino group bound to a pocket at one end of the groove and its terminal carboxyl group bound to a pocket at the other end of the groove.

Peptide binding motifs generally contain two to three anchor positions (Rammensee, Friede, and Stevanoviic 1995). Other features such as secondary anchors and disfavored residues have also been described as playing an important role in defining the peptide-MHC interaction (Ruppert et al. 1993). The peptide-binding cleft of HLA class II molecules is formed by the outer  $\alpha 1$  and  $\beta 1$  domains. Since it does not narrow at the ends, it can accommodate longer peptides containing up to 30 but usually 13 to 17 amino acids. The peptides presented by class II molecules are derived from extracellular proteins internalized by endocytosis and degraded in an endocytic compartment. Hence, the MHC class II-dependent pathway of antigen presentation is called the endocytic or exogenous pathway.

## 2.2 HLA nomenclature and typing methods

According to the World Health Organization (WHO) Committee on Nomenclature for Factors of the HLA System (Holdsworth et al. 2009), each HLA allele name has a unique number corresponding to up to four sets of digits separated by colons. The 2-digits before the first colon describe the type, which often corresponds to the serological antigen carried by an allotype. The next set of digits are used to list the subtypes, numbers being assigned in the order in which DNA sequences have been determined. Broad families of alleles are clustered into serotypes (e.g., HLA-A1).

There are two levels of typing: low-resolution (2-digits) and high-resolution (at least 4-digits). Low-resolution typing delivers results equivalent to serological typing and can be achieved by serological (microlymphocytotoxicity test) or molecular techniques. Due to its simplicity and low cost, serologic typing is still used in some laboratories. High-resolution typing can only be achieved by DNA-based techniques allowing classification of the individual alleles within each serotype (e.g., HLA-A\*01:01). A number of HLA typing methods based on PCR technology have been developed. PCR with sequence-specific primers (PCR-SSP), PCR followed by sequence-specific oligonucleotide probing (PCR-SSO) and PCR followed by sequencing-based typing (PCR-SBT) are currently the most commonly used molecular methods for low- and high-resolution HLA typing. These methods have displaced serology in most laboratories because of a much greater accuracy.

### 2.2.1 Types and nomenclature of HLA expression variants

To label HLA alleles with an alternative expression pattern the WHO Nomenclature Committee for Factors of the HLA System defined suffixes ('N', 'L', 'S', 'C', 'A', 'Q'), that are

added to an allele name to indicate its expression status (Holdsworth et al. 2009). Alleles shown to be not expressed ('Null' alleles) are given the suffix 'N'. The alteration does not necessarily imply the lack of production of an internal partial product which might be a T-cell target (Elsner and Blasczyk 2004). HLA alleles with 'Low' cell surface expression of an intact antigen compared to normal levels are indicated using the suffix 'L'. The suffix 'S' is used to denote an allele specifying a protein which is exclusively expressed as a 'Secreted' molecule but not as a cell surface protein. A 'Q' suffix is used when the expression of an allele is 'Questionable' given that the mutation seen in the allele has previously been shown to affect normal expression levels. The suffix 'C' is used to denote an allele product found in the 'Cytoplasm' but not on the cell surface, and the suffix 'A' indicates 'Aberrant' expression.

Currently, 197 HLA class I alleles (168 N, 5 L, 24 Q and 1 S allele) and 21 HLA class II alleles (all null alleles) with variant expression are listed in the IMGT/HLA database on the HLA nomenclature website ([www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla); released April 2011). As of April 2011, no alleles have been named with a 'C' or 'A' suffix. Most of these alleles carry mutations causing stop codons, leaving no doubt about their non-expression. Examples include HLA-A\*02:82N, HLA-A\*23:08N, HLA-A\*24:132N, HLA-B\*14:07N, HLA-B\*39:40N, HLA-B\*46:07N, HLA-B\*56:190N, or HLA-C\*06:49N. In the case of HLA-A\*03:03N, a frame deletion is responsible for non-expression (Lienert et al. 1996).

Only four HLA-A alleles (HLA-A\*01:01:38L, HLA-A\*02:01:01:02L, HLA-A\*24:02:01:02L, HLA-A\*30:14L) and one HLA-B allele (HLA-B\*39:01:01:02L) with low-expression patterns have been identified up to now (Balas et al. 1994; Magor et al. 1997; Laforet et al. 1997; Dunn et al. 2004; Hirv et al. 2006; Perrier et al. 2006). Low expression of these alleles is usually associated with a low expression of the corresponding mRNA. However, the alteration causing the low expression of HLA-A\*30:14L is not associated with a reduced mRNA level, but rather seems to result from the loss of the disulfide bond between the cysteine residues at positions 101 and 164 in the  $\alpha 2$  domain (Hirv et al. 2006; Hinrichs et al. 2009).

The only soluble secreted allele (S) known so far is HLA-B\*44:02:01:02S (Dubois et al. 2004). This HLA-B44 variant was typed as a null allele by microlymphocytotoxicity, whereas the B\*44:02:01:01 allele was identified by PCR-SSP. DNA sequencing revealed a single nucleotide difference at the end of intron 4 in the acceptor splicing site, leading to a splicing error characterized by the deletion of exon 5 (transmembrane domain of the HLA antigen).

All known HLA class I Q alleles (7 HLA-A, 9 HLA-B and 8 HLA-C) and the HLA-A\*30:14L allele have cysteine residue mutations at amino acid position 101 or 164 affecting the 101/164 disulfide bridge in the  $\alpha 2$  domain. Point mutations altering codon 101 have been described for HLA-C\*02:25Q and HLA-C\*03:22Q (Middleton et al. 2006). In the case of HLA-A\*02:293Q, HLA-A\*11:50Q, HLA-A\*30:14L (Hirv et al. 2006), HLA-A\*32:11Q (Tang et al. 2006), HLA-B\*15:218Q, HLA-B\*35:65Q (Elsner et al. 2006), HLA-B\*37:16Q, HLA-B\*39:38Q (Tang et al. 2006), HLA-B\*40:133Q, HLA-C\*04:59Q, HLA-C\*07:121Q, HLA-C\*12:42Q, HLA-C\*15:32Q and HLA-C\*16:16Q, point mutations in codon 164 result in a replacement of the Cys residue, causing disruption of the disulfide bond in the  $\alpha 2$  domain. HLA-A\*30:14L is the only one of these alleles described as having a low expression pattern not affecting the corresponding mRNA levels (Hirv et al. 2006; Hinrichs et al. 2009). There are no known alleles with an amino acid mutation at positions 203 or 259 affecting the bridge in the  $\alpha 3$  domain.



### 2.3 HLA in transplantation

The best donor is an HLA genotypically matched sibling identified by family typing. When no identical sibling donor is available, transplantation of stem cells from an HLA-matched unrelated donor can result in comparable disease-free survival, particularly for good-risk patients (Petersdorf et al. 2004; Petersdorf 2007; 2008). Nevertheless, unrelated transplantation is associated with a higher frequency of post-transplant complications than in genotypically matched sibling HSCT, mainly because of undefined HLA incompatibilities. The negative impact of HLA mismatches on the outcome of hematopoietic stem cell transplantation has been demonstrated in a variety of studies (Mickelson et al. 2000; Ottinger et al. 2003; Schaffer et al. 2003). Most allele mismatches affect differences in the T-cell receptor contact area of the heavy chain or the peptide-binding site causing a change in the peptide binding repertoire both leading to a T cell-mediated allorecognition.

*HistoCheck* ([www.histocheck.org](http://www.histocheck.org)) is an online tool which helps clinicians and researchers visualize the amino acid substitutions of HLA alleles so that they can make informed judgments about their functional similarity (Elsner et al. 2004). Because exact HLA matching is often not possible, it is important to understand which alleles are the most similar. *HistoCheck* provides crystallography-based 3-dimensional (3D) visualizations of the allelic mismatches by highlighting amino acid mismatches, positions, and functions. The user is provided with dissimilarity scores (DSSs) for the amino acids involved as well as an over-all DSS for the two alleles. However, scoring HLA mismatches by *HistoCheck* has not been shown to predict clinical outcome in unrelated hematopoietic stem cell transplantation.

Several large-scale studies have shown that high-resolution matching of patients and unrelated donors significantly improves post-transplant survival (Bray et al. 2008), the incidence and severity of acute and chronic GVHD (Morishima et al. 2002; Morishima et al. 2007), and engraftment (Petersdorf et al. 2001; Flomenberg et al. 2004; Lee et al. 2007; Petersdorf 2008). Regarding cord blood transplantation, several studies have shown that the degree of HLA match is important as well, but a large cell dose may be at least equally important (Laughlin et al. 2004; Rocha, Sanz, and Gluckman 2004; Arcese et al. 2006; Eapen et al. 2007).

The National Marrow Donor Program (NMDP, [www.marrow.org](http://www.marrow.org)) proposed minimum HLA matching requirements for adult donors for HLA-A, -B, -C and -DRB1 (8/8) typed, at high resolution by DNA-based methods and cord blood units (CBU) for HLA-A, -B, (low resolution) and -DRB1 (high resolution) (Table 1) (Bray et al. 2008; Kamani et al. 2008).

Considering HLA allele and haplotype frequencies can be very useful when interpreting typing results and finding appropriate donors. Simply knowing that a patient's haplotype is extremely rare can prevent futile registry searches. Considering allele frequency alone is insufficient, because a rare allele can be acceptable when it is found in its most common haplotype. Being aware of rare alleles and haplotypes is also an important factor in quality control. Furthermore, typing results in registries are often incomplete. In the case where there are two matching donors, but each donor typing is incomplete with respect to different alleles, then haplotype frequencies can help choose the donor who is most likely to be an exact match. To overcome these limitations the new matching algorithm *HapLogic* ([www.marrow.org](http://www.marrow.org)) and *Haplocheck* ([www.haplocheck.org](http://www.haplocheck.org)) were developed. *HapLogic* a new enhanced matching algorithm that automatically identifies the donors or CBUs with the highest potential to match the patient, was established by the NMDP to accelerate and improve the efficiency of searches. The new matching algorithm analyzes the haplotypes of

millions of donors on NMDP's *Be The Match Registry*. HapLogic uses advanced logic to predict a donor's or CBU's high-resolution match and builds upon mathematical formulas that predict DR match in AB donors (Hurley et al. 2006).

HLA locus	Tissue type patient?	Match donor and patient?
A	Yes, allele level	Yes
B	Yes, allele level	Yes
C	Yes, allele level	Yes
DRA	No	No
DRB1	Yes, allele level	Yes
DRB3, 4, and 5	Yes (DRB1 association)	Unknown
DQA1	No	No
DQB1	Yes (DRB1 association)	Uncertain
DPA1	No	No
DPB1	No	Uncertain

Table 1. HLA tissue typing recommended by the NMDP (from [www.marlow.org](http://www.marlow.org)) (Bray et al. 2008; Kamani et al. 2008)

The web tool *HaploCheck* is addressing this chance by ranking typing results based upon haplotype frequencies. The user enters the typing results for a patient, for which the cis/trans phase is unknown. The result is a list of separated haplotypes, ordered by frequency. Very rare alleles and associations are highlighted to inform the user of potential problems when searching registries, or to identify potential typing errors. For the case that a single mismatch is unavoidable, the user is presented with a list of mismatch-containing haplotypes and their frequencies. This can not only prevent futile registry searches, but also enable the clinician to make decisions about accepted mismatches before initiating a registry search.

### 3. Prevalence and allogenicity of HLA class I expression variants

Few investigators have systematically addressed the prevalence of HLA null and alternatively expressed alleles, which has been shown to be about 0.003% and 0.3%, respectively (Noreen et al. 2001; Elsner and Blasczyk 2004; Smith et al. 2005). Considering that most studies indicate that the prevalence of these alleles is around 1 per 1000 individuals, these alleles are not particularly rare. Consequently, it was recommended that laboratories typing unrelated bone marrow patients and donors should have a strategy to identify these expression variants (Elsner and Blasczyk 2004).

HLA null and expression variants are typically identified by the discrepancy between serological and molecular typing results. As molecular typing techniques have nearly displaced serological methods and are focusing on selected regions of the HLA genes many expression variants are likely to be overlooked. In solid organ transplantation, HLA expression variants are not considered in the matching procedure. In allogeneic HSCT, expression variants make an essential difference and can strongly affect transplant-related mortality since HLA mismatches are the major cause of severe GvHD or graft rejection. Thus, in contrast to solid organ transplantation, excluding HLA expression variants is required in the matching process for HSCT (Elsner and Blasczyk 2004; Hirv et al. 2006; Hinrichs et al. 2009).

Overlooking an HLA null allele in the donor would result in a T cell-mediated allorecognition of the recipient's HLA and may lead to the development of acute severe GvHD (Elsner and Blasczyk 2004). In the reverse setting (recipient null allele, donor expressed allele), allogeneic recognition of the recipient's stem cells may lead to their destruction and subsequent graft failure. Accordingly, mismatches between expressed and non-expressed HLA variants should be avoided in HSCT. In case of a recipient with an HLA null allele having no HLA-identical donor with the same null variant, matching must be performed as if the patient would be homozygous for the expressed allele of the respective HLA locus (Figure 1).

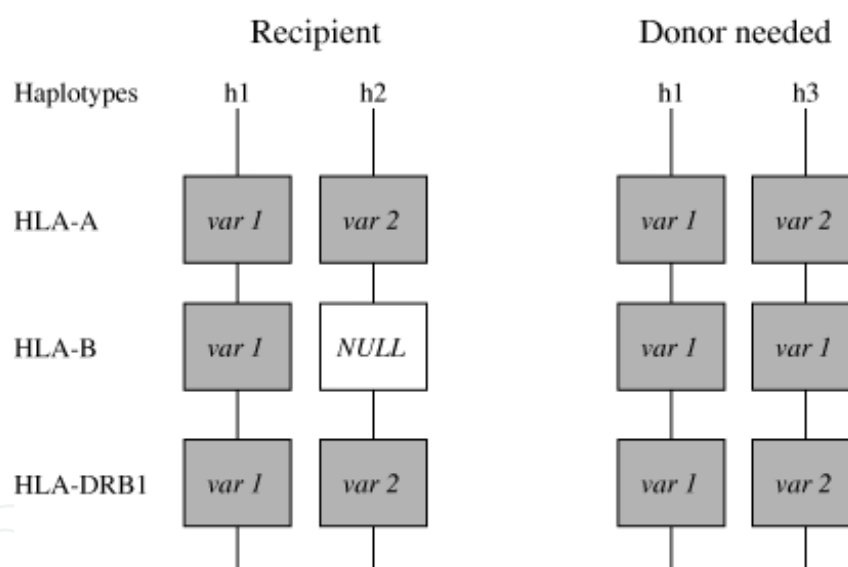


Fig. 1. Haplotypes in a recipient-donor combination with a null allele in one of the recipient's haplotypes. Shaded boxes indicate normally expressed variants. The recipient carries an HLA-B null allele (white box). In the donor search the recipient's haplotype h2 has to be 'replaced' by haplotype (h3) containing the expressed HLA-B allele (var1). However, such a haplotype may be rare and a matching donor hard to find (Elsner and Blasczyk 2004).

On the other hand, an incomplete HLA molecule may be generated, as has been shown for HLA-B\*44:02:01:02S, which might be presented via the indirect allogeneic recognition pathway (Magor et al. 1997; Dubois et al. 2004). Provided that the HLA-derived peptides fit into the peptide-binding groove and are capable of triggering a strong T-cell response, they may act as minor histocompatibility antigens (mHags). This could also apply to those HLA



expression variants where the transcription of a truncated mRNA is known and/or translation is probable. It also shows that premature stop codons do not automatically lead to the interruption of transcription (Balas et al. 1994; Laforet et al. 1997; Magor et al. 1997; Dunn et al. 2004; Hirv et al. 2006; Perrier et al. 2006; Eiz-Vesper, Blasczyk, and Horn 2007). In the light of countless non-HLA mHags this is probably of inferior importance.

#### **4. Characterization of HLA expression variants by cytokine-induced HLA secretion**

Because of the clinical importance of expression variants an HLA secretion assay was designed capable of discriminating between low-expression (L) and non-expressed (N) HLA variant alleles and assigning questionably expressed (Q) alleles to either group (Hinrichs et al. 2009).

All of the aforementioned HLA class I alleles with an unknown expression profile (Q alleles; 7 HLA-A, 9 HLA-B and 8 HLA-C) and HLA-A\*30:14L, have a mutation of cysteine residue 101 or 164 affecting disulfide bridge 101/164 in the  $\alpha 2$  domain. Because HLA-A\*30:14L is the only one of these alleles described to have a low expression pattern with no effect on mRNA levels (Hirv et al. 2006; Hinrichs et al. 2009), A\*30:14L was used as an expression model. HLA-A\*30:14L was reported to be non-expressed under normal conditions and to show weak aberrant expression after cultivation of the corresponding B-lymphoblastoid cell line at 30°C (Hirv et al. 2006).

HLA-A\*30:14L was originally identified in a patient suffering from chronic myeloid leukemia (Hirv et al. 2006). The sequence of this allele is identical to that of HLA-A\*30:01 except for a transversion at nucleotide position 563 in exon 3 (guanine to cytosine substitution), resulting in a replacement of cysteine by serine at position 164, impairing disulfide bridge formation in the  $\alpha 2$  domain of the mature polypeptide. This alteration of the secondary structure presumably decreases expression, rendering HLA-A\*30:14L basically undetectable by serology.

Human cell lines (HEK293, C1R and K562) expressing recombinant soluble HLA (sHLA) molecules (Table 2) were incubated with interferon (IFN)- $\gamma$  and/or tumor necrosis factor (TNF)- $\alpha$  (Hinrichs et al. 2009). These pro-inflammatory cytokines are known to enhance the expression of HLA molecules by affecting the interaction of DNA-binding proteins with the HLA-A promoter regions, resulting in the increased transcription of heavy and light chain genes (Girdlestone 1996; Gobin et al. 1997; Gobin et al. 1998; Gobin et al. 1999; Johnson 2003). In addition, these cytokines induce the transcription of proteasome subunits, peptide transporters and chaperones that promote the expression of HLA class I molecules by providing peptides for presentation (Ma et al. 1997; Lankat-Buttgereit and Tampe 2002).

Expression of soluble HLA-A\*30:14L and HLA-A\*30:01 was measured in the supernatants of transfected and untransfected cells incubated with or without IFN- $\gamma$  and/or TNF- $\alpha$  using a W6/32 and anti- $\beta 2$ -microglobulin-based sandwich ELISA (Figure 1) (Bade-Doeding et al. 2007). HLA-A\*30:14L was not detected in the supernatant of unstimulated transfectants. Stimulation with IFN- $\gamma$  and/or TNF- $\alpha$  increased HLA-A\*30:14L secretion to detectable levels and increased HLA-A\*30:01 expression up to 8-fold, but did not result in any difference between mRNA levels of HLA-A\*30:14L and A\*30:01 (Figure 2).

Day		Expression level (ng/ml)		
		HEK293	C1R	K562
1	HLA-A*30:01	31.3 ± 10.3	98.0 ± 14.7	155.8 ± 73.9
	HLA-A*30:14L	0	3.2 ± 1.7	0
3	HLA-A*30:01	383.2 ± 56.5	225.8 ± 177.5	143.5 ± 40.8
	HLA-A*30:14L	1.8 ± 1.4	0	0
7	HLA-A*30:01	160.4 ± 3.2	253.6 ± 16.1	175.9 ± 74.7
	HLA-A*30:14L	0	9.7 ± 4.9	0

Table 2. Soluble HLA-A\*30:01 and HLA-A\*30:14L expression levels (ng/ml) in the supernatant of three transfected cell lines (HEK293, C1R and K562) after 1, 3 and 7 days of incubation.

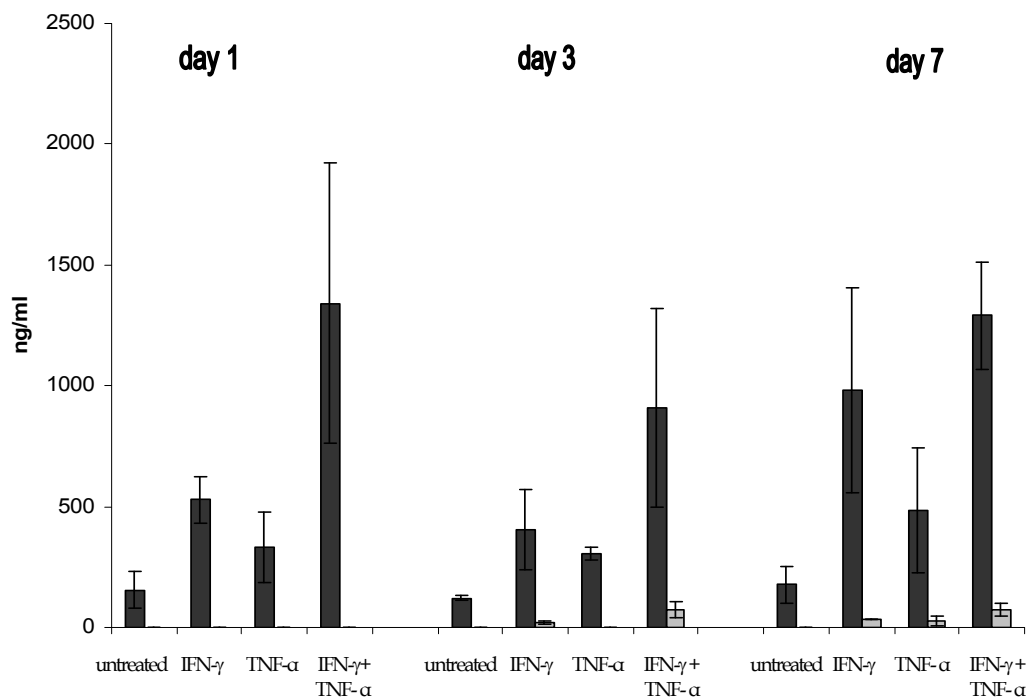


Fig. 2. Secretion of soluble HLA-A\*30:01 and HLA-A\*30:14L by transfected K562 cells

Expression of mRNA transcripts of both alleles was determined by real-time PCR. For control, Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell line (B-LCL) expressing HLA-A\*30:14L was established from cells of the patient's mother (genotype HLA-A\*30:14L,\*02:01) (Hirv et al. 2006). The positive control was a B-LCL expressing HLA-A\*30:01 (genotype HLA-A\*30:01,\*02:01). In both B-LCLs and HEK293 cells, the mRNA level of HLA-A\*30:14L was nearly identical to that of HLA-A\*30:01 (Figure 3). This finding suggests that the mRNA transcription rate of sHLA-A\*30:14L is not affected by the mutation at nucleotide position 563 (G->C). The mRNA levels of both alleles clearly increased in response to combined stimulation with IFN- $\gamma$  and TNF- $\alpha$ . In view of this lack of any difference in mRNA transcription, the protein expression defect is most likely caused by the missing disulfide bond in the  $\alpha$ 2 domain.

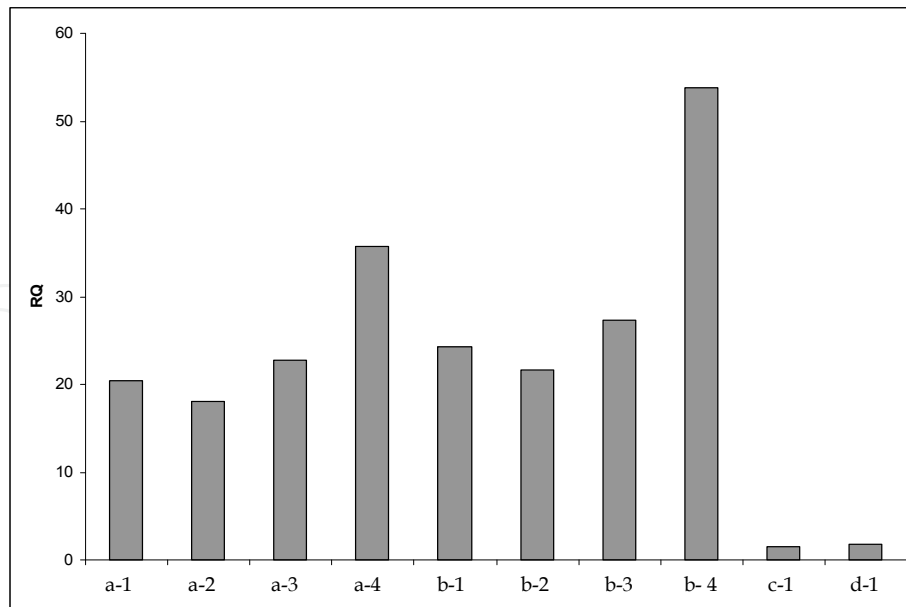


Fig. 3. Detection of mRNA levels of HLA-A\*30 alleles in B-LCLs and HEK293 cells

mRNA expression of the HLA-A\*30 alleles in transfected cell lines and B-LCLs was determined by real-time PCR. Shown are representative results for HEK293 cells measured after 3 days of culture in the presence ("treated") or absence ("untreated") of the cytokines IFN- $\gamma$  and/or TNF- $\alpha$ . Data were acquired using a probe specific for the HLA-A30 sequence. Similar results were achieved for all transfected cell lines. Lanes: a) sHLA-A\*30:01-transfected HEK293, b) sHLA-A\*30:14L-transfected HEK293, c) EBRCC-256 (HLA-A\*30:01), d) EBRCC-1818 (HLA-A\*30:14L), 1 untreated, 2 IFN- $\gamma$ -treated, 3 TNF- $\alpha$ -treated, 4 IFN- $\gamma$  plus TNF- $\alpha$ -treated

The observation that HLA-A\*30:14L protein accumulates inside the cells indicates that HLA-A\*30:14L translation is not affected. Consequently, the lack of protein secretion in the supernatant is best explained by post-translational instability of the HLA-A\*30:14L molecules because of the missing disulfide bridge (Hinrichs et al. 2009). Based on these findings, it is likely that the intracellular enriched HLA-A\*30:14L protein is a major substrate for proteasomal cleavage and that it provides a flood of peptide fragments presented to cytotoxic T lymphocytes. As a result of this indirect surface expression by the presentation of peptide fragments, it is possible that GvHD or graft rejection might be promoted in the event of mismatching (Benichou 1999). Consequently, considering HLA-A\*30:14L as null allele is, in case of a mismatch with any other HLA-A allele, potentially more dangerous in terms of GvHD and graft rejection than a mismatch with its most related allele HLA-A\*30:01. Indeed, mistyping HLA-A\*30:14L as an N allele has led to a severe GvHD in a patient transplanted with hematopoietic stem cells from an HLA-A\*02:01 homozygous donor (Hirv et al. 2006).

In recent studies, the cytokine-based HLA secretion assay was used to classify the expression patterns of HLA-A\*32:11Q (Tang et al. 2006) and HLA-B\*35:65Q (Elsner et al. 2006). Both alleles undergo cysteine substitution at amino acid position 164 and thus lack the disulfide bond between the cysteine residues at amino acid positions 101 and 164 in the  $\alpha 2$  domain of the mature protein. This interferes with HLA maturation inside the ER and therefore impairs cell surface expression. In concordance with the results of Hinrichs

et al. (Hinrichs et al. 2009), IFN- $\gamma$  and TNF- $\alpha$  increased the expression of the HLA expression variants, making HLA-A\*32:11Q and HLA-B\*35:65Q distinctly detectable. Compared to HLA-A\*32:01 and HLA-B\*35:01, the variants have very weak protein levels, indicating a low expression status. Consequently, they should be handled as low expression variants (L alleles).

## 5. The nature of peptides presented by HLA class I expression variants

The functional integrity of HLA low-expression variants is a prerequisite for considering them as essential in hematopoietic stem cell donor and recipient matching to diminish the risk of serious complications such as GvHD or graft rejection. HLA class I molecules present endogenous peptides 8-12 amino acids in length to CD8<sup>+</sup> cytotoxic T lymphocytes (Natarajan et al. 1999). Most amino acid polymorphisms of different HLA class I molecules are located in the peptide-binding region shaped by parts of the  $\alpha$ 1 and  $\alpha$ 2 domains; these polymorphisms determine the characteristics of presented peptides. Peptide motifs have been reported for the most common HLA-A and B alleles and for some rare variants. Importantly, differences in peptide binding among the alleles of a serological group have also been described (Prilliman et al. 1999; Bade-Doeding et al. 2011). Identification and comparison of allele-specific peptide-binding motifs provide important information for donor-recipient matching and prediction of HLA subtype allogenicity in allogeneic HSCT.

In order to determine the functionality of HLA low-expression alleles, peptides from recombinant truncated HLA-A\*30:14L molecules secreted in the supernatant of a human cell line were eluted and sequenced (Hinrichs et al 2010). The suitability of the monoclonal anti-HLA class I antibody W6/32 for purifying recombinant HLA-A\*30:14L molecules suggested its proper folding and assembly. Presumably, more soluble HLA-A\*30:14L is produced and secreted into the supernatant that might not be correctly folded because of the lack of a disulfide bridge in the  $\alpha$ 2 domain.

Edman pool sequencing of eluted peptides corroborated the hypothesis that peptides are presented by HLA low expression variants and showed identical peptide motifs in HLA-A\*30:01 and HLA-A\*30:14L confirming the previously described peptide motif of A\*30:01 (Lamberth et al. 2008; Sidney et al. 2008). The C-terminal position (P $\Omega$ ) was identified as a primary anchor position. The preferred residues of the HLA-A\*30 peptide epitopes at this position are lysine (K), valine (V) or arginine (R). The preference for lysine as the top amino acid at the P $\Omega$  position of the bound peptides, like described by positional scanning combinatorial peptide libraries (PSCPL) analysis, could be consolidated by the obtained peptide sequence data (Lamberth et al. 2008; Sidney et al. 2008). Position P3 of the peptides was identified as a primary-secondary anchor showing a high preference for the basic amino acids K and R. Six amino acids are reportedly favored at position P2: phenylalanine (F), serine (S), threonine (T), valine (V), isoleucine (I) or leucine (L).

The size of the obtained peptides ranged from 8 to 14 amino acids, but most had a length of 9 to 10 aa. The sequences of 200 HLA-A\*30:01 ligands and of 100 HLA-A\*30:14L ligands were identified. The following three peptide epitopes (3%) were presented by both HLA-A\*30:01 and HLA-A\*30:14L: 1) VLDTGGPPV, a nonameric peptide derived from titin (isoform N2-A, aa position 19783-19791), a protein of human muscle ultrastructure and

elasticity; 2) EITALAPSTMK, an 11-mer peptide derived from human muscle protein ACTA1 (actin, alpha 1, skeletal muscle; aa position 301-311); and 3) DNIQGITKPAIR, a 12-mer peptide derived from a histone protein (HIST2H4A; aa position 25-36) (Table 3).

Peptide position	1	2	3	4	5	6	7	8	9	10	11	12	Source
Ligand	V	L	D	T	P	G	P	P	V				Titin (TTN titin isoform N2-A)
	E	I	T	A	L	A	P	S	T	M	K		Actin (ACTA1)
	D	N	I	Q	G	I	T	K	P	A	I	R	Histone (HIST2H4A)

Table 3. Shared peptide epitopes of HLA-A\*30:14L and HLA-A\*30:01

To verify the presentation of naturally presented peptides from recombinant HLA-A\*30:01/30:14L molecules, peptide binding was analyzed by flow cytometry (Storkus et al. 1993; Zeh et al. 1994; Maeurer et al. 1996) in three EBV-transformed B-LCLs expressing either HLA-A\*30:14L,\*02:01 (Ulm-241539), HLA-A\*30:01,\*02:01 (EBRCC-256) or HLA-A\*02:01 (EBRCC-2296) (Warburton et al. 1994; Hirv et al. 2006; Hinrichs et al. 2009; 2010). Acid treatment of the cell lines resulted in the dissociation of the naturally bound peptides and the release of  $\beta$ 2 microglobulin from the HLA class I heavy chain. The HLA class I molecules were then reconstituted by adding fluorescein isothiocyanate (FITC)-labeled HLA peptide ligands and recombinant  $\beta$ 2 microglobulin. The synthetic FITC-labeled peptide EITALAK(FITC)PSTMK (HLA-A\*30:01/30:14L) and the immunodominant HLA-A\*02:01-restricted CMVpp65<sub>495-503</sub> peptide (NLVPMK(FITC)VATV) were used. Reconstitution of HLA with the HLA-A\*30 ligand mounted up to 51% (Ulm-241539) and 74% (EBRCC-256), respectively, compared to 25% for the HLA-A\*02:01 homozygous cell line (EBRCC-2296). Binding on cells expressing the normal HLA-A\*30:01 allele was higher than on those expressing HLA-A\*30:14L, the low expression variant (Figure 4). The results confirm that the A\*30 peptide previously isolated binds to HLA-A\*30 on the cell surface. Peptide binding was found for the A\*30:01 specific peptide on the HLA-A\*30:14L-expressing cell line (Ulm-241539), indicating the stability of HLA-A\*30:14L cell surface expression.

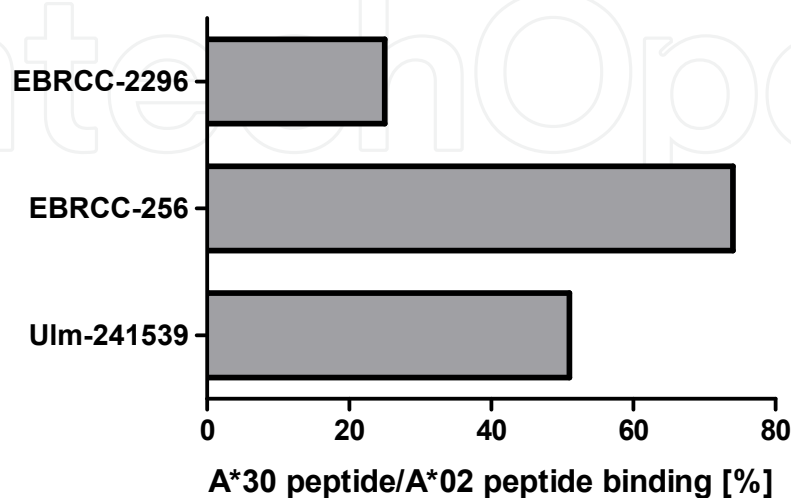


Fig. 4. Relative A\*30/A\*02:01 peptide-binding intensities for different HLA-expressing B-LCLs



Homology-based modeling for each HLA-A\*30 alleles with the shared 9, 11 and 12-mer peptide epitopes revealed only marginal differences between the two HLA-A\*30 alleles. The HLA-A\*30:01 and HLA-A\*30:14L models were essentially identical with the Cys164 Ser substitution, but simply adopted an alternate rotamer conformation upon breakage of the disulfide bond. Therefore, only the HLA-A\*30:14L model is illustrated (Figure 5).

Although the models look identical and the alleles appear to bind identical peptides, the Cys164Ser variation could potentially generate additional flexibility within the peptide-binding groove, thereby influencing binding kinetics, particularly in peptides of lower affinity. Such an effect could stimulate a T-cell immune response and have serious implications in allogeneic HSCT.

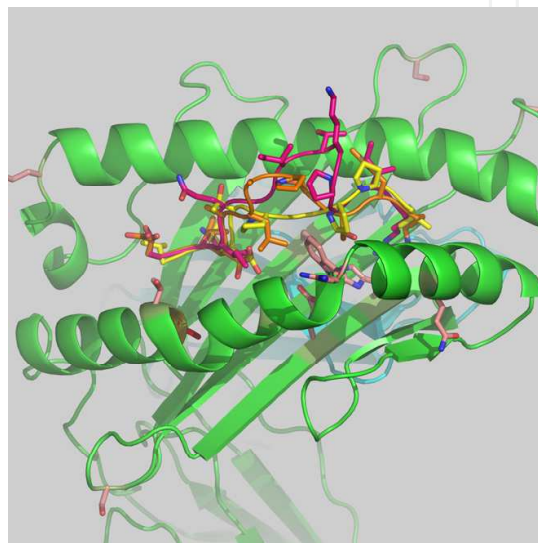


Fig. 5. Homology-based model of HLA-A\*30:14L with the three shared peptide ligands

Modeling of the HLA-A\*30:01 and HLA-A\*30:14L structures was carried out using the SCWRL homology-based modeling server (Wang, Canutescu, and Dunbrack 2008) while employing the crystal structure of the closely related HLA-A\*11:01 (1Q94) as a template. Peptide templates for 9-mer (1Q94), 11-mer (2BVO) and 12-mer (3BW9) were superimposed and merged with the HLA-A\*30:14L model. Peptide mutagenesis was then performed using DeepView (Guex and Peitsch 1997) and the rotamer library to find the best side chain orientations with minimum steric clashes. Each model was then subjected to energy minimization using DeepView software. The graphics program PyMOL (<http://www.pymol.org>) was used to generate the structural models.

## 6. Conclusions

Since HLA mismatches are the main cause of severe GvHD and graft rejection, misinterpretation of HLA null alleles and expression variants as irrelevant could strongly affect transplant-related mortality.

The cytokine-based HLA secretion assay can be used to distinguish between low-expressed and non-expressed HLA alleles in order to classify alleles with a currently undefined expression status (questionable alleles, Q) as well as to re-classify certain alleles which have been assigned as null variants (N). Additionally, discrimination between cytokine inducible and non-inducible defect alleles may be important in allotransplant settings in which a

cytokine storm usually occurs following pre-transplant myeloablative conditioning or post-transplant immunosuppressive therapy.

The fact that the monoclonal anti-HLA class I antibody W6/32 is a conformational antibody implies that only correctly folded,  $\beta 2$  microglobulin-assembled and peptide-loaded MHC complexes can be detected. This suggests that parts of soluble HLA-A\*30:14L molecules are assembled correctly and secreted by transfectants. Presumably, more soluble HLA-A\*30:14L is produced and secreted into the supernatant, but it might not be correctly folded because of the lack of the disulfide bridge in the  $\alpha 2$  domain. Therefore, these molecules are not detected by the conformational anti-HLA-ABC mAb. This assumption arose after comparing mRNA and associated protein levels of HLA-A\*30:14L and HLA-A\*30:01 alleles (Hinrichs et al. 2009). Additionally, it was found that HLA-A\*30:14L accumulates inside the cells; therefore, it might be a major substrate for proteasomal cleavage and could provide a flood of peptide fragments presented to cytotoxic T lymphocytes. As a result of this indirect allorecognition pathway, GvHD or graft rejection might be promoted in the event of a severe mismatch.

It was shown for the first time that an HLA low expression allele (HLA-A\*30:14L) presents peptides with identical features to those of its most closely related relative, HLA-A\*30:01 (Hinrichs et al. 2010). The results indicate that a mismatch at amino acid position 164 might be permissive. Therefore, mismatching of these alleles will presumably be of low allogenicity in allogeneic HSCT. The fact that a low expression variant is not only functional and able to present peptides, but also shares epitopes with its related variant leads to the conclusion that low expression variants need to be considered in donor selection as permissive or non-permissive mismatches, respectively. Increasing knowledge of the expression behavior of HLA expression variants, such as L and Q alleles, will help to improve HLA allogenicity prediction algorithms by delivering proof that these variants are fully functional. Taking all relevant factors into account, the results shown allow to predict the immunogenicity of aberrantly expressed alleles in a transplant setting.

In the case of HLA-A\*30:14L misinterpreting it as a null allele is, in case of a mismatch with any other HLA-A allele, potentially more dangerous in terms of GvHD and graft rejection according to the direct and indirect allo-recognition pathway than a mismatch with its most related allele HLA-A\*30:01. Indeed, mistyping HLA-A\*30:14L as an N allele has led to a severe GvHD in a patient transplanted with hematopoietic stem cells from an HLA-A\*02:01 homozygous donor (Hirv et al. 2006).

In order to predict the relevance of similar alleles with disulfide bridge rearrangements (e.g., HLA-A\*32:11Q and B\*35:65Q) in allogeneic HSCT, it is important to know their surface expression as well as their peptide binding of HLA variants. From a clinical perspective, HLA variants with similar disulfide bridge variations need to be considered as functionally active in an allogeneic HSCT setting as long as the opposite has not been shown.

## 7. Acknowledgments

The authors would like to thank Jan Hinrichs and Daniel Föll for performing the experiments and contributing helpful discussions. The authors are grateful to Christina Bade-Döding, Nektarios Ladas and Trevor Huyton for help in peptide data analysis and homology-based modeling. This work is supported in part by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence

REBIRTH (From Regenerative Biology to Reconstructive Therapy) and by the German Federal Ministry of Education and Research (reference number: 01EO0802).

## 8. References

- Arcese, W., V. Rocha, M. Labopin, G. Sanz, A. P. Iori, M. de Lima, A. Sirvent, A. Busca, S. Asano, I. Ionescu, P. Wernet, and E. Gluckman. 2006. Unrelated cord blood transplants in adults with hematologic malignancies. *Haematologica* 91 (2):223-30.
- Bade-Doeding, C., A. Theodossis, S. Gras, L. Kjer-Nielsen, B. Eiz-Vesper, A. Seltsam, T. Huyton, J. Rossjohn, J. McCluskey, and R. Blasczyk. 2011. The impact of human leukocyte antigen (HLA) micropolymorphism on ligand specificity within the HLA-B\*41 allotypic family. *Haematologica* 96 (1):110-8.
- Bade-Doeding, C., D. S. DeLuca, A. Seltsam, R. Blasczyk, and B. Eiz-Vesper. 2007. Amino acid 95 causes strong alteration of peptide position Pomega in HLA-B\*41 variants. *Immunogenetics* 59 (4):253-9.
- Balas, A., F. Garcia-Sanchez, F. Gomez-Reino, and J. L. Vicario. 1994. HLA class I allele (HLA-A2) expression defect associated with a mutation in its enhancer B inverted CAT box in two families. *Hum Immunol* 41 (1):69-73.
- Benichou, G. 1999. Direct and indirect antigen recognition: the pathways to allograft immune rejection. *Front Biosci* 4:D476-80.
- Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329 (6139):512-8.
- Bjorkman P.J., M. A. Saper , B. Samraoui , W. S. Bennett, J. L.Strominger, and D. C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329 (6139):506-12.
- Bray, R. A., C. K. Hurley, N. R. Kamani, A. Woolfrey, C. Muller, S. Spellman, M. Setterholm, and D. L. Confer. 2008. National marrow donor program HLA matching guidelines for unrelated adult donor hematopoietic cell transplants. *Biol Blood Marrow Transplant* 14 (9 Suppl):45-53.
- Chicz, R. M., R. G. Urban, J. C. Gorga, D. A. Vignali, W. S. Lane, and J. L. Strominger. 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J Exp Med* 178 (1):27-47.
- Dubois, V., J. M. Tiercy, M. P. Labonne, A. Dormoy, and L. Gebuhrer. 2004. A new HLA-B44 allele (B\*44020102S) with a splicing mutation leading to a complete deletion of exon 5. *Tissue Antigens* 63 (2):173-80.
- Dunn, P. P., J. R. Turton, J. Downing, S. Williams, C. V. Navarrete, and C. Darke. 2004. HLA-A\*24020102L in the UK blood donor population. *Tissue Antigens* 63 (6):589-91.
- Eapen, M., P. Rubinstein, M. J. Zhang, C. Stevens, J. Kurtzberg, A. Scaradavou, F. R. Loberiza, R. E. Champlin, J. P. Klein, M. M. Horowitz, and J. E. Wagner. 2007. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet* 369 (9577):1947-54.
- Eiz-Vesper, B., R. Blasczyk, and P. A. Horn. 2007. Description of the first HLA-DRB1 null allele. *Immunogenetics* 59 (6):507-10.
- Elsner, H. A., and R. Blasczyk. 2004. Immunogenetics of HLA null alleles: implications for blood stem cell transplantation. *Tissue Antigens* 64 (6):687-95.

- Elsner, H. A., D. DeLuca, J. Strub, and R. Blasczyk. 2004. HistoCheck: rating of HLA class I and II mismatches by an internet-based software tool. *Bone Marrow Transplant* 33 (2):165-9.
- Elsner, H. A., P. A. Horn, C. Schoenemann, W. W. Altermann, and R. Blasczyk. 2006. Aberrant expression of HLA-B\*3565Q is associated with a disrupted disulfide bond. *Immunogenetics* 58 (11):929-31.
- Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H. G. Rammensee. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351 (6324):290-6.
- Ferrari, G., D. D. Kostyu, J. Cox, D. V. Dawson, J. Flores, K. J. Weinhold, and S. Osmanov. 2000. Identification of highly conserved and broadly cross-reactive HIV type 1 cytotoxic T lymphocyte epitopes as candidate immunogens for inclusion in Mycobacterium bovis BCG-vectored HIV vaccines. *AIDS Res Hum Retroviruses* 16 (14):1433-43.
- Flomenberg, N., L. A. Baxter-Lowe, D. Confer, M. Fernandez-Vina, A. Filipovich, M. Horowitz, C. Hurley, C. Kollman, C. Anasetti, H. Noreen, A. Begovich, W. Hildebrand, E. Petersdorf, B. Schmeckpeper, M. Setterholm, E. Trachtenberg, T. Williams, E. Yunis, and D. Weisdorf. 2004. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood* 104 (7):1923-30.
- Garrett, T. P., M. A. Saper, P. J. Bjorkman, J. L. Strominger, and D. C. Wiley. 1989. Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* 342 (6250):692-6.
- Girdlestone, J. 1996. Transcriptional regulation of MHC class I genes. *Eur J Immunogenet* 23 (5):395-413.
- Gobin, S. J., V. Keijsers, M. van Zutphen, and P. J. van den Elsen. 1998. The role of enhancer A in the locus-specific transactivation of classical and nonclassical HLA class I genes by nuclear factor kappa B. *J Immunol* 161 (5):2276-83.
- Gobin, S. J., A. Peijnenburg, V. Keijsers, and P. J. van den Elsen. 1997. Site alpha is crucial for two routes of IFN gamma-induced MHC class I transactivation: the ISRE-mediated route and a novel pathway involving CIITA. *Immunity* 6 (5):601-11.
- Gobin, S. J., M. van Zutphen, A. M. Woltman, and P. J. van den Elsen. 1999. Transactivation of classical and nonclassical HLA class I genes through the IFN-stimulated response element. *J Immunol* 163 (3):1428-34.
- Guex, N., and M. C. Peitsch. 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 18 (15):2714-23.
- Haselden, B. M., A. B. Kay, and M. Larche. 2000. Peptide-mediated immune responses in specific immunotherapy. *Int Arch Allergy Immunol* 122 (4):229-37.
- Hinrichs, J., C. Figueiredo, K. Hirv, J. Mytilineos, R. Blasczyk, P. A. Horn, and B. Eiz-Vesper. 2009. Discrimination of HLA null and low expression alleles by cytokine-induced secretion of recombinant soluble HLA. *Mol Immunol* 46 (7):1451-7.
- Hinrichs, J., D. Foll, C. Bade-Doeding, T. Huyton, R. Blasczyk, and B. Eiz-Vesper. 2010. The nature of peptides presented by an HLA class I low expression allele. *Haematologica* 95 (8):1373-80.



- Hirv, K., U. Pannicke, J. Mytilineos, and K. Schwarz. 2006. Disulfide bridge disruption in the alpha2 domain of the HLA class I molecule leads to low expression of the corresponding antigen. *Hum Immunol* 67 (8):589-96.
- Holdsworth, R., C. K. Hurley, S. G. Marsh, M. Lau, H. J. Noreen, J. H. Kempenich, M. Setterholm, and M. Maiers. 2009. The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens* 73 (2):95-170.
- Hurley, C. K., J. E. Wagner, M. I. Setterholm, and D. L. Confer. 2006. Advances in HLA: practical implications for selecting adult donors and cord blood units. *Biol Blood Marrow Transplant* 12 (1 Suppl 1):28-33.
- Jardetzky, T. S., W. S. Lane, R. A. Robinson, D. R. Madden, and D. C. Wiley. 1991. Identification of self peptides bound to purified HLA-B27. *Nature* 353 (6342):326-9.
- Johnson, D. R. 2003. Locus-specific constitutive and cytokine-induced HLA class I gene expression. *J Immunol* 170 (4):1894-902.
- Kamani, N., S. Spellman, C. K. Hurley, J. N. Barker, F. O. Smith, M. Oudshoorn, R. Bray, A. Smith, T. M. Williams, B. Logan, M. Eapen, C. Anasetti, M. Setterholm, and D. L. Confer. 2008. State of the art review: HLA matching and outcome of unrelated donor umbilical cord blood transplants. *Biol Blood Marrow Transplant* 14 (1):1-6.
- Laforet, M., N. Froelich, A. Parissiadis, H. Bausinger, B. Pfeiffer, and M. M. Tongio. 1997. An intronic mutation responsible for a low level of expression of an HLA-A\*24 allele. *Tissue Antigens* 50 (4):340-6.
- Lamberth, K., G. Roder, M. Harndahl, M. Nielsen, C. Lundegaard, C. Schafer-Nielsen, O. Lund, and S. Buus. 2008. The peptide-binding specificity of HLA-A\*3001 demonstrates membership of the HLA-A3 supertype. *Immunogenetics* 60 (11):633-43.
- Lankat-Buttgereit, B., and R. Tampe. 2002. The transporter associated with antigen processing: function and implications in human diseases. *Physiol Rev* 82 (1):187-204.
- Laughlin, M. J., M. Eapen, P. Rubinstein, J. E. Wagner, M. J. Zhang, R. E. Champlin, C. Stevens, J. N. Barker, R. P. Gale, H. M. Lazarus, D. I. Marks, J. J. van Rood, A. Scaradavou, and M. M. Horowitz. 2004. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 351 (22):2265-75.
- Lee, S. J., J. Klein, M. Haagenson, L. A. Baxter-Lowe, D. L. Confer, M. Eapen, M. Fernandez-Vina, N. Flomenberg, M. Horowitz, C. K. Hurley, H. Noreen, M. Oudshoorn, E. Petersdorf, M. Setterholm, S. Spellman, D. Weisdorf, T. M. Williams, and C. Anasetti. 2007. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 110 (13):4576-83.
- Lienert, K., G. Russ, S. Lester, G. Bennett, X. Gao, and J. McCluskey. 1996. Stable inheritance of an HLA-"blank" phenotype associated with a structural mutation in the HLA-A\*0301 gene. *Tissue Antigens* 48 (3):187-91.
- Ma, W., P. J. Lehner, P. Cresswell, J. S. Pober, and D. R. Johnson. 1997. Interferon-gamma rapidly increases peptide transporter (TAP) subunit expression and peptide transport capacity in endothelial cells. *J Biol Chem* 272 (26):16585-90.
- Madden, D. R., J. C. Gorga, J. L. Strominger, and D. C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* 353 (6342):321-5.



- Maeurer, M. J., D. Martin, E. Elder, W. J. Storkus, and M. T. Lotze. 1996. Detection of naturally processed and HLA-A1-presented melanoma T-cell epitopes defined by CD8(+) T-cells' release of granulocyte-macrophage colony-stimulating factor but not by cytolysis. *Clin Cancer Res* 2 (1):87-95.
- Magor, K. E., E. J. Taylor, S. Y. Shen, E. Martinez-Naves, N. M. Valiante, R. S. Wells, J. E. Gumperz, E. J. Adams, A. M. Little, F. Williams, D. Middleton, X. Gao, J. McCluskey, P. Parham, and K. Lienert-Weidenbach. 1997. Natural inactivation of a common HLA allele (A\*2402) has occurred on at least three separate occasions. *J Immunol* 158 (11):5242-50.
- Matsumura, M., D. H. Fremont, P. A. Peterson, and I. A. Wilson. 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* 257 (5072):927-34.
- Mickelson, E. M., E. Petersdorf, C. Anasetti, P. Martin, A. Woolfrey, and J. A. Hansen. 2000. HLA matching in hematopoietic cell transplantation. *Hum Immunol* 61 (2):92-100.
- Middleton, D., A. Meenagh, S. G. Marsh, and J. Martin. 2006. A HLA-Cw\*03 allele, Cw\*0322Q with limited or no expression. *Tissue Antigens* 67 (4):343-5.
- Morishima, Y., T. Kawase, M. Malkki, and E. W. Petersdorf. 2007. Effect of HLA-A2 allele disparity on clinical outcome in hematopoietic cell transplantation from unrelated donors. *Tissue Antigens* 69 Suppl 1:31-5.
- Morishima, Y., T. Sasazuki, H. Inoko, T. Juji, T. Akaza, K. Yamamoto, Y. Ishikawa, S. Kato, H. Sao, H. Sakamaki, K. Kawa, N. Hamajima, S. Asano, and Y. Kodera. 2002. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood* 99 (11):4200-6.
- Natarajan, K., H. Li, R. A. Mariuzza, and D. H. Margulies. 1999. MHC class I molecules, structure and function. *Rev Immunogenet* 1 (1):32-46.
- Noreen, H. J., N. Yu, M. Setterholm, M. Ohashi, J. Baisch, R. Endres, M. Fernandez-Vina, U. Heine, S. Hsu, M. Kamoun, Y. Mitsuishi, D. Monos, L. Perlee, S. Rodriguez-Marino, A. Smith, S. Y. Yang, K. Shipp, J. Hegland, and C. K. Hurley. 2001. Validation of DNA-based HLA-A and HLA-B testing of volunteers for a bone marrow registry through parallel testing with serology. *Tissue Antigens* 57 (3):221-9.
- Ottinger, H. D., S. Ferencik, D. W. Beelen, M. Lindemann, R. Peceny, A. H. Elmaagacli, J. Husing, and H. Grosse-Wilde. 2003. Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. *Blood* 102 (3):1131-7.
- Parham, P., C. E. Lomen, D. A. Lawlor, J. P. Ways, N. Holmes, H. L. Coppin, R. D. Salter, A. M. Wan, and P. D. Ennis. 1988. Nature of polymorphism in HLA-A, -B, and -C molecules. *Proc Natl Acad Sci U S A* 85 (11):4005-9.
- Perrier, P., A. Dormoy, C. Andre-Botte, and N. Froelich. 2006. HLA-A\*02010102L: a laborious assignment. *Tissue Antigens* 68 (5):442-5.
- Petersdorf, E. W. 2007. Risk assessment in haematopoietic stem cell transplantation: histocompatibility. *Best Pract Res Clin Haematol* 20 (2):155-70.
- Petersdorf, E. W. 2008. Optimal HLA matching in hematopoietic cell transplantation. *Curr Opin Immunol* 20 (5):588-93.

- Petersdorf, E. W., C. Anasetti, P. J. Martin, T. Gooley, J. Radich, M. Malkki, A. Woolfrey, A. Smith, E. Mickelson, and J. A. Hansen. 2004. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. *Blood* 104 (9):2976-80.
- Petersdorf, E. W., J. A. Hansen, P. J. Martin, A. Woolfrey, M. Malkki, T. Gooley, B. Storer, E. Mickelson, A. Smith, and C. Anasetti. 2001. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. *N Engl J Med* 345 (25):1794-800.
- Prilliman, K. R., K. W. Jackson, M. Lindsey, J. Wang, D. Crawford, and W. H. Hildebrand. 1999. HLA-B15 peptide ligands are preferentially anchored at their C termini. *J Immunol* 162 (12):7277-84.
- Rammensee, H. G., T. Friede, and S. Stevanović. 1995. MHC ligands and peptide motifs: first listing. *Immunogenetics* 41 (4):178-228.
- Rocha, V., G. Sanz, and E. Gluckman. 2004. Umbilical cord blood transplantation. *Curr Opin Hematol* 11 (6):375-85.
- Rudensky, AYu, P. Preston-Hurlburt, S. C. Hong, A. Barlow, and C. A. Janeway, Jr. 1991. Sequence analysis of peptides bound to MHC class II molecules. *Nature* 353 (6345):622-7.
- Ruppert, J., R. T. Kubo, J. Sidney, H. M. Grey, and A. Sette. 1994. Class I MHC-peptide interaction: structural and functional aspects. *Behring Inst Mitt* (94):48-60.
- Ruppert, J., J. Sidney, E. Celis, R. T. Kubo, H. M. Grey, and A. Sette. 1993. Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell* 74 (5):929-37.
- Sant, A. J. 1994. Endogenous antigen presentation by MHC class II molecules. *Immunol Res* 13 (4):253-67.
- Schaffer, M., A. Aldener-Cannava, M. Remberger, O. Ringden, and O. Olerup. 2003. Roles of HLA-B, HLA-C and HLA-DPA1 incompatibilities in the outcome of unrelated stem-cell transplantation. *Tissue Antigens* 62 (3):243-50.
- Sette, A., S. Buus, E. Appella, J. A. Smith, R. Chesnut, C. Miles, S. M. Colon, and H. M. Grey. 1989. Prediction of major histocompatibility complex binding regions of protein antigens by sequence pattern analysis. *Proc Natl Acad Sci U S A* 86 (9):3296-300.
- Sette, A., S. Buus, S. Colon, J. A. Smith, C. Miles, and H. M. Grey. 1987. Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. *Nature* 328 (6129):395-9.
- Sette, A., J. Sidney, M. F. del Guercio, S. Southwood, J. Ruppert, C. Dahlberg, H. M. Grey, and R. T. Kubo. 1994. Peptide binding to the most frequent HLA-A class I alleles measured by quantitative molecular binding assays. *Mol Immunol* 31 (11):813-22.
- Sidney, J., E. Assarsson, C. Moore, S. Ngo, C. Pinilla, A. Sette, and B. Peters. 2008. Quantitative peptide binding motifs for 19 human and mouse MHC class I molecules derived using positional scanning combinatorial peptide libraries. *Immunome Res* 4:2.
- Singh, R. R. 2000. The potential use of peptides and vaccination to treat systemic lupus erythematosus. *Curr Opin Rheumatol* 12 (5):399-406.
- Smith, D. M., J. E. Baker, W. B. Gardner, G. W. Martens, and E. D. Agura. 2005. HLA class I null alleles and new alleles affect unrelated bone marrow donor searches. *Tissue Antigens* 66 (2):93-8.

- Solheim, J. C. 1999. Class I MHC molecules: assembly and antigen presentation. *Immunol Rev* 172:11-9.
- Storkus, W. J., H. J. Zeh, 3rd, R. D. Salter, and M. T. Lotze. 1993. Identification of T-cell epitopes: rapid isolation of class I-presented peptides from viable cells by mild acid elution. *J Immunother Emphasis Tumor Immunol* 14 (2):94-103.
- Tang, T. F., L. Hou, B. Tu, W. Y. Hwang, A. E. Yeoh, J. Ng, and C. K. Hurley. 2006. Identification of nine new HLA class I alleles in volunteers from the Singapore stem cell donor registries. *Tissue Antigens* 68 (6):518-20.
- Wang, E., G. Q. Phan, and F. M. Marincola. 2001. T-cell-directed cancer vaccines: the melanoma model. *Expert Opin Biol Ther* 1 (2):277-90.
- Wang, Q., A. A. Canutescu, and R. L. Dunbrack, Jr. 2008. SCWRL and MolIDE: computer programs for side-chain conformation prediction and homology modeling. *Nat Protoc* 3 (12):1832-47.
- Warburton, R. J., M. Matsui, S. L. Rowland-Jones, M. C. Gammon, G. E. Katzenstein, T. Wei, M. Edidin, H. J. Zweerink, A. J. McMichael, and J. A. Frelinger. 1994. Mutation of the alpha 2 domain disulfide bridge of the class I molecule HLA-A\*0201. Effect on maturation and peptide presentation. *Hum Immunol* 39 (4):261-71.
- Yewdell, J. W., and J. R. Bennink. 2001. Cut and trim: generating MHC class I peptide ligands. *Curr Opin Immunol* 13 (1):13-8.
- Yewdell, J. W., C. C. Norbury, and J. R. Bennink. 1999. Mechanisms of exogenous antigen presentation by MHC class I molecules in vitro and in vivo: implications for generating CD8+ T cell responses to infectious agents, tumors, transplants, and vaccines. *Adv Immunol* 73:1-77.
- Zeh, H. J., 3rd, G. H. Leder, M. T. Lotze, R. D. Salter, M. Tector, G. Stuber, S. Modrow, and W. J. Storkus. 1994. Flow-cytometric determination of peptide-class I complex formation. Identification of p53 peptides that bind to HLA-A2. *Hum Immunol* 39 (2):79-86.

IntechOpen



## **New Advances in Stem Cell Transplantation**

Edited by Prof. Taner Demirer

ISBN 978-953-51-0013-3

Hard cover, 582 pages

**Publisher** InTech

**Published online** 24, February, 2012

**Published in print edition** February, 2012

This book documents the increased number of stem cell-related research, clinical applications, and views for the future. The book covers a wide range of issues in cell-based therapy and regenerative medicine, and includes clinical and preclinical chapters from the respected authors involved with stem cell studies and research from around the world. It complements and extends the basics of stem cell physiology, hematopoietic stem cells, issues related to clinical problems, tissue typing, cryopreservation, dendritic cells, mesenchymal cells, neuroscience, endovascular cells and other tissues. In addition, tissue engineering that employs novel methods with stem cells is explored. Clearly, the continued use of biomedical engineering will depend heavily on stem cells, and this book is well positioned to provide comprehensive coverage of these developments.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Britta Eiz-Vesper and Rainer Blasczyk (2012). Relevance of HLA Expression Variants in Stem Cell Transplantation, *New Advances in Stem Cell Transplantation*, Prof. Taner Demirer (Ed.), ISBN: 978-953-51-0013-3, InTech, Available from: <http://www.intechopen.com/books/new-advances-in-stem-cell-transplantation/relevance-of-hla-expression-variants-in-stem-cell-transplantation>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen