Recognition targets of Vδ2^{-} γδ T cells

- Shared activating ligands in cancer and CMV infection? –

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General information

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Abstract

Recently, Vδ2$^-$ γδ T cells have been shown to be reactive against both cytomegalovirus (CMV)-infected cells and tumor cells. This dual reactivity can be important for clinical application after allogeneic stem cell transplantation, since CMV-infection causes morbidity and mortality in these patients. The dual reactive Vδ2$^-$ γδ T cells could be interesting as a treatment for these patients, since these cells are known to kill tumor cells, while at the same time control CMV infection and reactivation. The dual reactivity of Vδ2$^-$ γδ T is most likely caused by antigens expressed on both tumor and CMV-infected cells. Until now, only a few antigens are identified for γδ T cells which are mainly recognized by Vδ2$^+$ γδ T cells. This review presents possible antigens for Vδ2$^-$ γδ T cells which might underlie the dual reactivity of these T cells. This review is focused on the MHC superfamily, since known ligands of γδ T cells are members of this family, and on tumor antigens, which are known to be overexpressed by tumors. Most of the found possible candidates were tumor antigens, like heat shock proteins (HSP60 and HSP70), adhesion molecules (ICAM-1, CD44, and β1 integrins), and IFITM1. Besides tumor antigens, also some members of the MHC superfamily are considered to be possible antigens underlying the dual reactivity, namely MICA*008 and EPCR.

Introduction

T cells are an important feature of the adaptive immune system and can be distinguished in T cells expressing the αβ T cell receptor (TCR) and T cells expressing the γδ TCR. γδ T cells represent only 1-5% of T lymphocytes in the peripheral blood and lymphoid organs, although in other anatomic locations they constitute a larger amount (10-50%) of the total number of T cells (Carding et al. 2002). Outside the circulating blood, γδ T cells are mainly present in epithelial-rich tissues, including skin, lung, and intestine (Deusch et al. 1991).

Although originating from the same thymocyte progenitor cells, αβ and γδ T cells fundamentally differ in a number of ways. Most strikingly, γδ T cells show characteristics of the innate immune system. For example, the γδ TCR is considered to function like a pattern recognition receptor (PRR) such as a toll-like receptor, resembling the antigen recognition mechanism of the innate immunity (Bonneville et al. 2010). The epithelial localization of γδ T cells also makes them a potent part of the host first defense, like the innate immune system. Finally, γδ T cells have been shown to possess antigen-presenting cell (APC) functions, like presenting antigens on MHC class II molecules, activating dendritic cells, and inducing proliferation and differentiation of naive $CD4^+$ and $CD8^+$ αβ T cells (Brandes et al. 2005; Devilder et al. 2006). Once activated however, γδ T cells are virtually indistinguishable from αβ T cells. For example, γδ T cells are able to lyse target cells through death receptors, like the FAS-receptor and TRAIL-receptor (Qin et al. 2009), release of cytotoxic effector molecules, like perforins and granzymes (Viey et al. 2005; Qin et al. 2009), and producing and secreting cytokines (Raga et al. 2003). Moreover, γδ T cells are capable of expanding clonally and

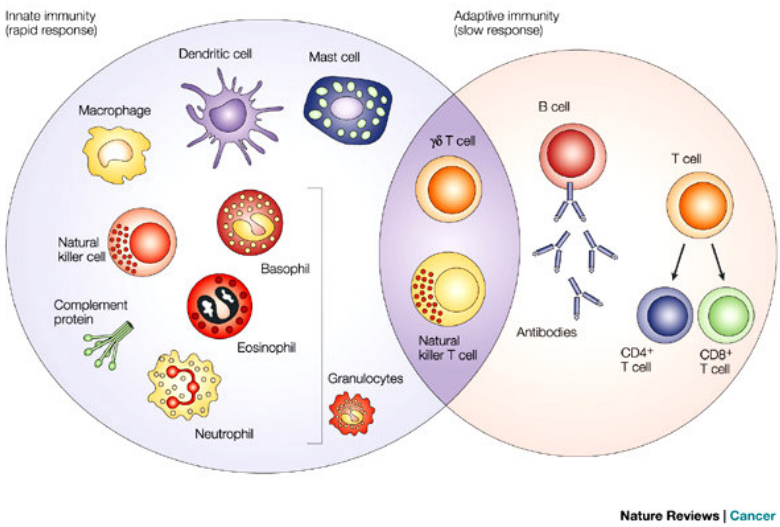

Figure 1 – γδ T cells are a link between the innate and adaptive immunity. γδ T cells have characteristics of both the innate and adaptive immune system, leadsing to the view that these cells form a bridge between those immune systems. (Dranoff 2004)

forming a memory pool, similar to αβ T cells (Martino et al. 2007). Taken together, through these characteristics, γδ T cells are considered a bridge between innate and adaptive immunity (Figure 1).

Although antigen recognition by the γδ TCR is believed to occur through innate-like pattern recognition mechanisms, γδ T cells have an enormous potential for TCR diversity, enabled by somatic recombination of a variable (V) gene segment with a joining (J) gene segment and with a diversity (D) gene segment. Including or excluding nucleotides at the joining sites of the gene segments further increases the TCR diversity (Carding et al. 2002). According to their γ- and δ-chains, different subsets of γδ T cells are distinguished, and nomenclature follows the identity of the V gene segments. To date, up to 14 different Vγ gene segments (Vγ1 to Vγ14) and 8 different Vδ gene segments (Vδ1 to Vδ8) have been identified, although for some segments functional expression in γδ T cells remains to be demonstrated. The subtypes of γδ T cells differ per anatomic location. For example, in the blood of healthy individuals, the Vγ9Vδ2 T cells represent ~90% of the γδ T cell population. In the intestine however, intraepithelial γδ T cells express mostly the Vδ1 gene associated with different Vγ genes (De Rosa et al. 2001).

The pathological contexts in which involvement of γδ T cells have been reported are numerous. γδ T cells play a role in the defense against certain viruses. Specifically, γδ T cells have been shown to participate in the immune response against human immunodeficiency virus (HIV), hepatits C virus (HCV), and the cytomegalovirus (CMV) (Sindhu et al. 2003). During viral infections, the number of γδ T cells in the blood and in the infected organs of the patient may be increased significantly (Sindhu et al. 2003). Some studies found that during HIV infection the number of Vδ2- γδ T cells expand, while the number of $V\delta2^+$ γδ T cells decrease (De Maria et al. 1992; Sindhu et al. 2003). Unfortunately, it has been shown that particularly $V\delta2^+$ T cells show reactivity towards HIV-infected cells (Wallace et al. 1996; Poccia et al. 1999). Also infection with the flavivirus HCV causes an increase in Vδ2- γδ T cells and a decrease in $V\delta2^+$ T cells, indicating that $V\delta2^-$ γδ T cells participate more in the immune response against HCV than $V\delta2^+$ T cells (Agrati et al. 2001; Par et al. 2002). γδ T cells are also known to expand after CMV infection. After infection, the virus remains in a latent state in the body and becomes reactivated in immunocompromised individuals. The expanded γδ T cells after reactivation of the virus consisted only of Vδ1 and Vδ3 T cells, meaning that the $V\delta2^+$ γδ T cells did not react to CMV-infection (Dechanet et al. 1999). The study of Couzi *et al.* showed that these $V\delta2^-$ γδ T cells are reactive against CMV-infect cells (Couzi et al. 2010).

γδ T cells have also been shown to be reactive against a wide variety of tumor cells *in vitro* and *in vivo*. γδ T cells have been isolated from tumor-infiltrating lymphocytes (TIL), and these γδ T cells recognize and kill the cells of the tumor from which they were isolated (Kabelitz et al. 2007). However, it has been shown that these γδ T cells can also recognize and kill cells of related tumors, while showing low reactivity towards non-transformed cells (Corvaisier et al. 2005; Viey et al. 2005).

Vδ2$^-$ γδ T cells have been shown to infiltrate epithelial tumors, including colon adenocarcinomas, melanomas, renal carcinomas, and lung carcinomas (Choudhary et al. 1995; Maeurer et al. 1996), (Zocchi et al. 1994; Bialasiewicz et al. 1999). Reactivity against hematological cancers was proposed to be a unique property of Vδ2$^+$ γδ T cells, however it has recently been shown that Vδ2$^-$ γδ T cells may also be reactive against leukemia and lymphoma (Meeh et al. 2006). Vδ2$^+$ γδ T cells have also been shown to recognize and kill epithelial tumors (Wrobel et al. 2007), indicating that there are no distinct anti-tumor specificities for the γδ T cell subtypes.

Interestingly, γδ T cells have been identified that are capable of reacting against both CMV-infected cells and tumor cells. The study of Halary *et al.* showed that a variety of Vδ2$^-$ γδ T cell clones were able to lyse CMV-infected fibroblasts and that these cells could also recognize intestinal tumor epithelial cells (Halary et al. 2005). This dual reactivity could be important for the understanding of the antigen recognition by γδ T cells. It may also be important for clinical applications of γδ T cells, for example after allogeneic stem cell transplantation (allo-SCT) where CMV infection or reactivation is a cause of morbidity and mortality after transplantation. In 60-70% of the CMV-seropositive patients receiving an allo-SCT, CMV reactivation occurs during this period (Hebart et al. 2004). The prognosis for a CMV infection after allo-SCT is poor, so defense against viral reactivation is crucial. In this respect, γδ T cells that possess dual reactivity against CMV-infected cells and tumor cells are interesting, since these T cells could kill leukemia cells while at the same time control the CMV infection, leading to a better prognosis after allo-SCT.

To understand the mechanism of the dual reactivity of γδ T cells, it is important to establish the antigens which cause this dual reactivity. As mentioned above, the recognition of antigens by γδ T cells differs in a fundamental way from αβ T cells. In contrast to αβ T cells, antigen recognition by γδ T cells resembles that of the innate immune system, allowing γδ T cells to recognize a broad range of antigens. Moreover, activation of the γδ TCR is not dependent on antigen presentation through classical MHC molecules, Although only a limited number of γδ T cell antigens have been identified so far, the general view is that γδ T cells recognize stress-related surface antigens on cancerous or infected cells (Kabelitz et al. 2007). Studies aimed at identifying γδ T cell antigens have so far focused mainly on Vδ2$^+$ γδ T cells (Figure 2). These T cells have been found to recognize phosphoantigens, which are phosphorylated intermediates of the non-mevalonate pathway of bacterial isoprenoid biosynthesis. Isopentenyl pyrophosphate (IPP) is an example of a phosphoantigen. Also mitochondrial F1-ATPase-related structure and delipidated apolipoprotein A1 are recognized by the Vδ2$^+$ γδ T cells (Kabelitz et al. 2007). In addition, members of the MHC superfamily, like MHC class I-related chains A and B (MICA and MICB) and UL-16 binding proteins (ULBPs), are known antigens for both Vδ2$^-$ and Vδ2$^+$ γδ T cells (Kabelitz et al. 2007). However, little is known about the antigens which

are recognized by Vδ2$^-$ γδ T cells, let alone which antigen(s) might mediate dual reactivity with CMV-infected cells and tumor cells.

In this review I aim to identify candidate antigens that may underlie the dual reactivity of Vδ2$^-$ γδ T cells. First, I focus on the MHC superfamily, since the known ligands of γδ T cells, like MHC class I-related chains and ULBPs, are members of this family. I also explore literature on tumor antigens, since this field harbors extensive knowledge on tumor-specific antigens. The expression of these molecules on tumor cells and on CMV-infected cells is investigated and the reactivity of γδ T cells to these molecules is described.

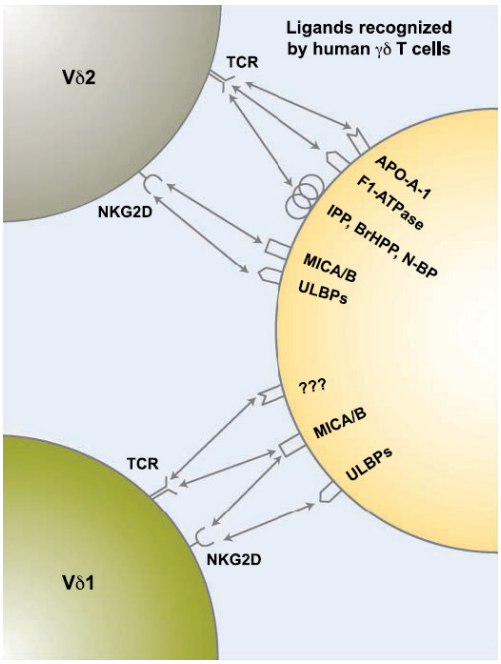

Figure 2 – Antigens of γδ T cells. Vδ2$^+$ γδ T cells are known to bind phosphoantigens, like isopentenyl pyrophosphate (IPP). Also mitochondrial F1-ATPase-related structure (F1-ATPase) and delipidated apolipoprotein A1 (APO-A-1) are recognized via the TCR receptor. Via the NKG2D receptor MICA, MICB and ULBPs are recognized. Vδ1$^+$ γδ T cells also have the NKG2D, which binds to MICA, MICB and ULBPs. Other ligands of the Vδ1$^+$ γδ T cells are yet to be established. Adapted from (Kabelitz et al. 2007)

MHC superfamily

Introduction

The MHC superfamily consists of the MHC class II molecules, classical MHC class I genes and non-classical MHC class I genes (Hughes et al. 1989; Duprat et al. 2006). The non-classical MHC class I molecules are MHC class I-like proteins, for example MHC class I-related chain (MIC) proteins, CD1 proteins and HFE proteins. The classical genes distinguish themselves from non-classical genes by their highly polymorphic characteristics. In humans more than 800 MHC class I alleles were identified, although the overall structure is similar (Robinson et al. 2009). The peptide binding groove of these molecules contains the majority of the allelic variations (Bjorkman et al. 1987). The structure of the classical MHC class I consist of multiple domains, namely three extracellular domains (α1, α2, and α3), a transmembrane domain, and a cytoplasmic tail. On the cell surface the molecules pair with the soluble protein β2-microglobulin (Figure 3A) (Bjorkman et al. 1987; Hughes et al. 1989). Although there is homology between the members of the MHC superfamily, the structure of non-classical MHC class I proteins is more divers. Not all of these proteins contain all three of the extracellular domains and only some of the non-classical proteins pair with β2-microglobulin (Figure 3) (Blumberg 1998) .

The functions of the classical and non-classical MHC class I proteins are very divers. Classical MHC class I protein are responsible for presenting antigens to immune cells and non-classical MHC class I proteins have more divers functions, however, some of these proteins, like CD1, MIC proteins and ULBPs, can also influence the immune system (Braud et al. 1999). CD1 proteins can, for example,

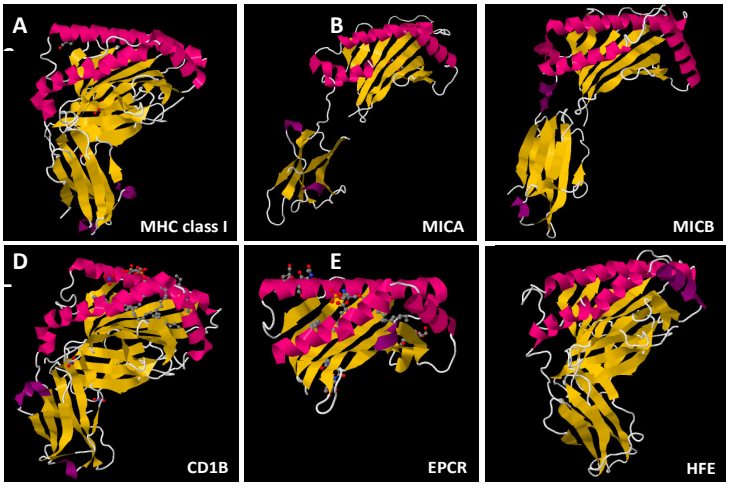

Figure 3 – Structure of members of the MHC superfamily. Homology can been seen between these molecules, mainly in the extracellular domains. These proteins, MHC class I (A), MICA (B), MICB (C), CD1B (D), EPCR (E), and HFE (F) all belongs to the MHC superfamily. Adapted from NCBI.

present lipid antigens and MIC proteins and ULBP are ligands for the receptor NKG2D expressed on certain immune cells (Hughes et al. 1989; Braud et al. 1999).

Since most of the γδ T cell antigens identified so far are members of the MHC superfamily (i.e. MICA/B, CD1, ULBPs and EPCR; see below), additional antigens may very well be found within this superfamily, including those that may confer γδ T cell dual reactivity (Kabelitz et al. 2007).

MHC class I-related chain proteins

This family contains 2 MHC class I-related chain (MIC) proteins, namely MICA and MICB. As stated above, the structure of these proteins is similar to that of MHC class I molecules, since it has also three external domains (α1-3), a transmembrane domain, and a cytoplasmic domain (Figure 3) (Stephens 2001). The main differences between these proteins and classical MHC class I molecules are that these MIC proteins do not associate with the β2-microglobulin and can not present antigens. The genes of the MIC proteins contain a lot of polymorphisms, there are 51 MICA alleles and 13 MICB alleles found. Some polymorphisms causes a less or higher capacity of the protein to bind to its receptor, NKG2D. Within different populations, the MICA allele frequencies differ. MICA*008, however, was found to be an allele that is common within all different populations (Stephens 2001). This allele encodes for a MICA variant arisen from a premature stop codon in the transmembrane region (Figure 4). While the transmembrane and cytoplasmic domains differ from the full length protein, the extracellular domains are still similar to that of the full length protein. It was found by Ashiru *et al.* that this truncated variant of MICA can still be functional (Ashiru et al. 2010).

On stressed or transformed cells MIC proteins appear on the cell surface, indicating that these proteins are markers of stress. These proteins are not found to be expressed on $CD4^+$ and $CD8^+$ αβ T cells and B cells (Zwirner et al. 1999). In cells of other organs, like thymus, liver, lung, MIC mRNA is found in the cytoplasm, although there is no expression of these proteins at the cell surface (Leelayuwat et al. 1994).

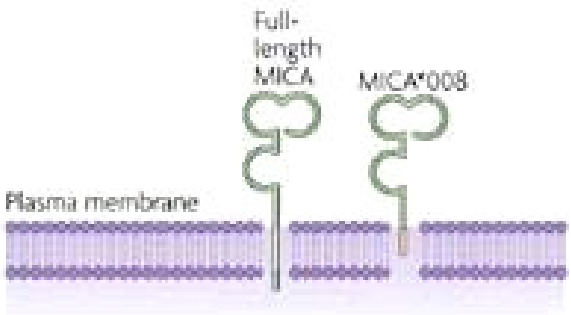

Figure 4 – Structure MICA and MICA*008. The truncated form of MICA, named MICA*008, has a short transmembrane domain and is misses the cytoplasmic domain compared to the full length MICA protein. Adapted from (Eagle et al. 2007)

As mentioned above, the receptor of MIC proteins is NKG2D, which is found to be expressed on natural killer (NK) cells, γδ T cells, and $CD8^+$ αβ T cells. It was demonstrated that Vδ1 γδ T cells can bind both MIC proteins expressed on gastric epithelia, meaning that γδ T cells can function through this receptor and its ligand (Groh et al. 1998). MICA and MICB were found to be expressed on several epithelial tumors isolated from the breast, lung, ovary, prostate, colon and kidney ,although both proteins were also found to be expressed on leukemic cells (Groh et al. 1999; Pende et al. 2002; Boissel et al. 2006; Kato et al. 2007). In these tumors, a high infiltration was found of Vδ1 γδ T cells, which have a high reactivity for cell lines expressing MICA and MICB (Groh et al. 1999; Qi et al. 2003).

Viral infections were found to actively downregulate of the MIC proteins on the cell surface (Chen et al. 2006; Richard et al. 2010). CMV also downregulates the expression of MIC proteins on the cell surface to evade the immune system. MICB was found to be downregulated by CMV, via the viral protein UL16. This protein binds to MICB in the endoplasmic reticulum (ER) causing sequestration of this molecule (Collins 2004). CMV has also another mechanism to suppress MICB cell surface expression, namely miRNA. MicoRNA-UL112, produced by CMV, was found to bind to MICB mRNA and thereby block the translation of the mRNA (Stern-Ginossar et al. 2007; Nachmani et al. 2010). In addition, UL142, another protein produced by the virus, is able to downregulate the cell surface expression of MICA sufficiently to protect against NK cell cytotoxicity (Chalupny et al. 2006; Ashiru et al. 2009). UL142 was found to be localized in the ER and the *cis*-Golgi apparatus, where it retains the full-length MICA in the *cis*-Golgi apparatus. Intriguingly, truncated MICA*008 is not retained by this viral protein and is still expressed at the cell surface (Figure 5). As mentioned above, there is a difference in the transmembrane and cytoplasmic domain of this truncated variant of MICA, although it has been shown that this variant is still functional (Collins 2004; Ashiru et al. 2010). Ashiru *et al.* show that MICA*008 is also produced on tumor cells and that these tumor cells release MICA*008 on exosomes (Ashiru et al. 2010). The MICA*008 on these exosomes was found to be functional and could bind the receptor NKG2D. The binding of this protein on exosomes to its receptor on NK cells promotes downregulation of the surface expression of this receptor, promoting tumor evasion (Ashiru et al. 2010).

Taken together, MIC proteins are known ligands for γδ T cells and are expressed on stressed cells, like infected and transformed cells. After infection of cells with CMV, there is downregulation of these proteins by the virus, indicating that these proteins may not be principal candidates as antigen causing the dual reactivity of $V\delta2^-$ γδ T cells. Interestingly, the most common allele of MICA, MICA*008, is not downregulated by CMV. These variant is also present on tumor cells, although the antigen secretion via exosomes means that the expression on the tumor cells is decreased. There is, however, still expression on the tumor cells. Hence, MICA*008 may be a valuable candidate for the γδ T cells dual-reactivity antigen.

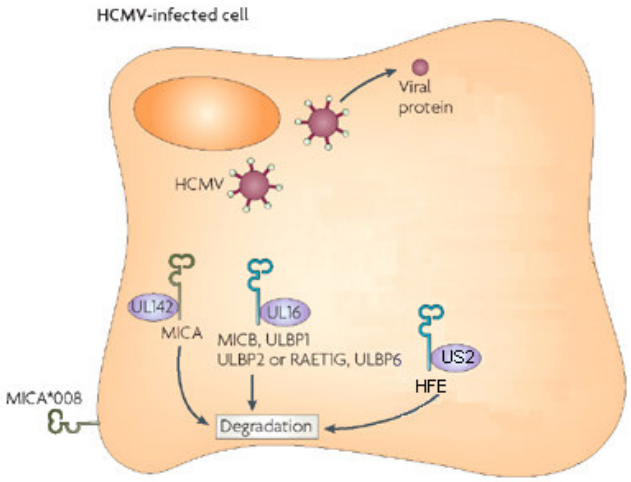

Figure 5 – Effect of CMV on members of MHC superfamily. This figure shows the inhibition of cell surface expression of some of the members of the MHC superfamily. MICA is suppressed by the viral protein UL142. UL16 binds and inhibits MICB, ULBP1, ULBP2, and ULBP6. The protein US2 has known to inhibit HFE expression. Adapted from (Lanier 2008).

CD1 proteins

The CD1 protein family also belongs to the MHC superfamily and contains 5 proteins, which are MHC class I-like glycoproteins. This CD1 family can be distinguished into two groups based on amino acid homology, expression patterns and functional attributes. Group 1 includes CD1a, CD1b, CD1c, CD1e and group 2 includes CD1d (Brutkiewicz 2006). Both groups have shown to be capable of presenting non-peptidic ligands, including lipids (Moody et al. 2005). CD1 proteins consist of three extracellular domains (α1-3), a transmembrane domain, and a cytoplasmic domain. CD1 molecules bind non-covalently to β2-microglobulin on the cell surface, which stabilizes the molecule (Figure 3; reviewed in (Moody et al. 2005)). All CD1 molecules contain a hydrophobic ligand binding site, which is loaded with specific self or non-self lipids in the endocytic compartments (Moody et al. 2005).

CD1 molecules are found to be expressed by cortical thymocytes and by cells of the myeloid lineage (Exley et al. 2000; Moody 2006). For example, CD1d is found on most cells of hematopoietic origin, namely dendritic cells, B cells and monocytes, however they are also found on non-professional APC, like intestinal and bile duct epithelial cells, keratinocytes, hair follicles, and peripheral nerve Schwann cells (Bonish et al. 2000; Exley et al. 2000; Fais et al. 2004; Adly et al. 2005). Also on these non-professional APC, CD1d molecules seem to have a role in the local immunity (Adly et al. 2005).

Concerning CD1 expression in tumor cells conflicting results are reported in literature. For example, Zheng *et al.* determined that in B cell chronic lymphocytic leukemia (B-CLL) CD1c and CD1d genes are downregulated, leading to evasion of the immune response (Zheng et al. 2002). On the contrary, the study of Fais *et al*. shows that there is overexpression of CD1d on B-CLL cells, which are

able to present lipid antigens to T cells (Fais et al. 2004). The rol of CD1 molecules on other tumor cells has not been established yet.

Unfortunately, CMV manipulates also the expression of CD1 molecules to avoid the immune system. CD1a is found to be downregulated in dendritic cells infected with CMV (Hertel et al. 2003). Also the study of Raferty *et al.* shows that there are multiple CMV-encoded mechanisms for CD1 blockage, which affect both the transcription and the cell surface expression of the CD1 molecules (Raftery et al. 2008). They found that cmvIL-10, a homologue of IL-10 produced by CMV, is responsible for the transcriptional downregulation of CD1 genes. Moreover, Raftery and colleagues found that there was at least one other gene or mechanism responsible for CD1 blockade post-transcriptionally distinct from the MHC class I blockade mechanism. This blockade could explain the found intracellular accumulation of CD1 molecules (Raftery et al. 2008). Which gene or mechanism is responsible for this post-transcriptional blockade is still not been identified.

Summarizing, CD1 molecules were considered possible candidates as shared antigen for both tumor and CMV-infected cells, since it has been found that γδ T cells can be CD1-reactive, indicating that CD1 proteins play a role in the antigen recognition of these T cells (Russano et al. 2007). However, the fact that CMV can downregulate these molecules and that these molecules are not overexpressed on many different types of tumors indicates that CD1 molecules may not be the principal antigen candidates explaining the dual reactivity of $V\delta2^-$ γδ T cells.

Endothelial cell protein C receptor

The endothelial cell protein C receptor (EPCR) is an endothelial membrane glycoprotein, which can bind protein C and activated protein C (APC) (Fukudome et al. 1994). The structure of EPCR resembles that of other members of the MHC superfamily, however, the α3-domain is absent and it does not associate with β2-microglobulin. Besides the α1- and α2-extracellular domains, the receptor has also a transmembrane domain and a small cytoplasmic tail (Figure 3; (Fukudome et al. 1994)). Like other proteins of the MHC superfamily EPCR has a deep groove which could be used for antigen presentation. This protein, however, does not present antigens, although it has like CD1 molecules a lipid molecule in this groove, which maintains the EPCR structure. This lipid is usually a phospholipid, namely phosphatidylcholine or phosphatidyl-ethanolamine (Oganesyan et al. 2002). EPCR is found mainly expressed on endothelial cells of larger vessels, although the receptor is also found on liver sinusoidal endothelium and in the spleen (Laszik et al. 1997). The receptor has been shown to play an anticoagulant role by improving protein C activation (Centelles et al. 2010). Blocking EPCR, using a monoclonal antibody, was found to accelerate arterial thrombus formation *in vivo*, indicating that EPCR plays an important role in the prevention of thrombus formation (Centelles et al. 2010). Besides membrane-bound EPCR also soluble EPCR was detected showing also has affinity for protein C and

APC. This soluble variant is nearly identical in size to the full length EPCR and is generated by shredding of the plasma membrane. Soluble EPCR was found to bind activated neutrophils, which contains specific binding sites for soluble EPCR (Kurosawa et al. 2000). The function of this soluble EPCR is to block protein C activation and the APC anticoagulant activity (Kurosawa et al. 2000).

Although EPCR is expressed mainly on endothelial cells of larger vessels, there was also expression found on the cell surface of different tumors, including melanoma, renal-, colon carcinomas, and leukemia (Tsuneyoshi et al. 2001; Scheffer et al. 2002). Overexpression of EPCR could indicate that EPCR contribute to pathogenesis and progression of certain cancers (Tsuneyoshi et al. 2001). Moreover, it was found that APC-EPCR interaction could also inhibit p53-mediated apoptosis, indicating that the high expression of EPCR on tumor cells might contribute to apoptosis inhibition in tumors (Wang et al. 2005).

During CMV-infection of endothelial cells upregulation of EPCR on the cell surface was also found (Willcox et al. 2010). Recently, it has been shown that EPCR can be an antigen for γδ T cells, especially Vδ2$^-$ γδ T cells (Willcox et al. 2010). They used Vγ4Vδ5 clones, and generated a monoclonal antibody which stained the targets cells and blocked the TCR-mediated recognition of tumor cells and CMV-infected cells. EPCR was found to be recognized by these clones and using surface plasmon resonance experiments they demonstrated specific binding of soluble EPCR to recombinant Vγ4δ5-TCR (Willcox et al. 2010).

Taken together, EPCR could be a molecule that may underlie the dual reactivity of Vδ2$^-$ γδ T cells, since overexpression of EPCR was found on the cell surface of both CMV-infected cells and tumor cells. Both cell types were already demonstrated to be killed by EPCR-reactive Vδ2$^-$ γδ T cells, making them interesting candidates for the dual reactivity.

UL16-binding proteins

The UL16-binding proteins (ULBPs, also called retinoic acid early transcripts, encoded by RAET1 genes) form a family that consists of 10 gene members, six of which encode potentially functional glycoproteins: ULBP1 (RAET1I), ULBP2 (RAET1H), ULBP3 (RAET1N), ULBP4 (RAET1E), ULBP5 (RAET1G), and ULBP6 (RAET1L) (Bacon et al. 2004; Cao et al. 2004; Eagle et al. 2009b). These proteins are MHC class I-like proteins, which lack the α3 domain and do not associate with β2-microglobulin. ULBP1-3 and ULBP6 are glycosylinositol phospholipid (GPI)-linked glycoproteins and ULBP4 and ULBP5 are transmembrane glycoproteins.

There are two alternative transcript found of ULBP5, namely RAET1G1 and RAET1G2, which are formed by alternative splicing of the transcript. RAET1G1 is the full length transcript with two extracellular domains (α1 and α2), a transmembrane domain and a cytoplasmic domain (Bacon et al.

2004; Eagle et al. 2009b). RAET1G2 is a truncated transcript lacking the transmembrane and cytoplasmic domain and is the soluble form of ULBP5 (Eagle et al. 2009b).

All ULBPs are poorly expressed on normal tissue and the expression of these proteins is induced by stress or infection (Eagle et al. 2006). A high expression of ULBPs is found in different cancer-derived cell lines and primary cancers, especially on T cell leukemia cell lines and lymphoid leukemia cells, although they were found also found on other tumors, like colon carcinoma, ovarian carcinoma and cervical carcinoma (Cao et al. 2004). RAET1G1 was also found on kidney renal cell carcinoma, lung adenocarcinoma, liver hepatoma, and skin squamous cell carcinoma (Eagle et al. 2009a).

ULBP proteins are ligands for the NKG2D receptor present on for example NK cells and γδ T cells. These proteins can trigger a NK effector response leading to the lysis of target cells. Poggi *et al.* demonstrate that ULBP3 can be recognized by Vδ1 γδ T cells (Poggi et al. 2004). They found that ULBP3 was upregulated in B-CLL cells after which the cells were lysed by the Vδ1 γδ T cells. Unfortunately, tumor cells have an escape mechanism for the immune system, namely producing soluble ULBPs, which occupy the NKG2D receptors on the immune cells and downregulate the expression of ULBPs on the cell surface (Bacon et al. 2004). One soluble form of ULBPs is formed by alternative splicing of the ULBP5 transcript (RAET1G2) and the other soluble form is produced by cleaving of the anchored ULBP2 by metalloproteinases (Waldhauer et al. 2006).

ULBP expression is upregulated after stress, suggesting that these proteins are also upregulated after CMV infection. This virus, however, can downregulate the cell surface expression of ULBPs and thereby create an escape mechanism for the immune system. CMV produces, for example, the protein UL16, which can bind to ULBP1, ULBP2, and ULBP6, causing sequestration of these proteins in the ER (Odeberg et al. 2003; Wilkinson et al. 2008). ULBP3 has also been found to be intracellularly retained in the *cis*-Golgi complex by CMV using the protein UL142, (Bennett et al. 2010). This downregulation of the ULBP3 surface expression was found to be sufficient to protect the infected cells against NK cell-mediated lysis. ULBP4 expression was found not to be induced by CMV infection, meaning that the CMV virus does not have to downregulate this molecule (Eagle et al. 2006).

In summary, the facts that the tumor cells secrete soluble ULBPs, downregulating the cell surface expression, and that CMV downregulates the cell surface expression of ULBP indicate that these molecules may not mediate the dual reactivity of $V\delta2^-$ γδ T cells and thereby may not be principal shared antigen candidates.

HFE protein

The HFE protein is a glycoprotein which has a similar structure like other MHC class I-like molecules. It contains three extracellular domains (α1-3), a transmembrane domain and a cytoplasmic domain and it also associates with β2-microglobulin. Compared to MHC class I molecules the binding groove is narrowed, which prohibits peptide binding in this groove. This protein is known to bind to the transferrin receptor (TfR), reducing iron uptake (Braud et al. 1999).

It was suggested that HFE was recognized by γδ T cells, because of the hepatic iron overload in δ-chain TCR knock-out mice (Ten Elshof et al. 1999). It seems that γδ T cells play a role in the regulation of iron absorption by binding to HFE with their TCR (Ten Elshof et al. 1999). This might suggest that γδ T cells can interact with HFE. HFE proteins have been found expressed on tumor cell lines, for example on meningiomas (Pascolo et al. 2005; Hanninen et al. 2009). CMV can, however, downregulate HFE expression by targeting the protein to the proteasome-mediated degradation pathway by the vial protein US2 (Ben-Arieh et al. 2001).

Taken together, the downregulation of HFE by CMV suggests that the protein could be recognized by the immune system, and that decreasing HFE expression is a way of CMV to avoid the immune system. However, this downregulation suggests that this protein may not be a principal candidate causing the dual reactivity of $V\delta2^-$ γδ T cells.

Tumor antigens

Introduction

Tumor antigens are molecules produced by tumor cells, triggering an immune response. They can be distinguished into two classes, namely tumor-associated and tumor-specific antigens. The main difference between these two classes is their expression pattern. Tumor-associated antigens are expressed on both tumor and some normal cells, while tumor-specific antigens are only expressed on tumor cells (Lucas et al. 2008). This is, however, not the most perfect classification, since most tumor-specific antigens have been shown to be expressed on normal cells (Coulie et al. 2001).

There are multiple methods developed to identify tumor antigens (Figure 6). One method is comparing proteins, peptides, and mRNA isolated from tumor cells with those isolated from healthy cells, using proteomics, mass spectrometry, or expression profiling. Based on the data from these experiments, a prediction can be made about possible tumor antigens (Stevanovic 2002). Another method is identifying epitopes recognized by tumor-reactive cytotoxic T lymphocytes or antibodies (Stevanovic 2002; Lucas et al. 2008).

Since the classification of tumor-specific and tumor-associated is not perfect, a new classification was made (Lucas et al. 2008). The tumor antigens can be distinguished in antigens resulting from mutations (unique antigens), shared tumor-specific antigens, differentiation antigens, and overexpressed antigens (Coulie et al. 2001). Unique antigens are antigens encoded by genes with somatic mutations and already 50 different kinds of unique antigens are described (Lucas et al. 2008). These somatic mutations consist mainly of mutated amino acid (60%), chimeric peptides of fused translocated genes (20%), and frameshifts. Shared tumor-specific antigens are proteins

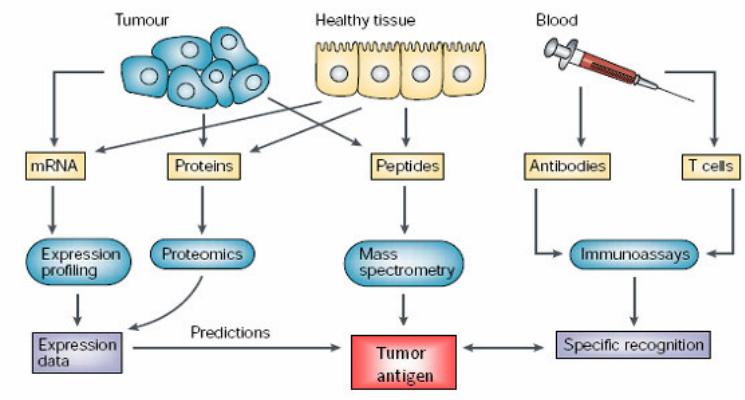

Figure 6 – Methods to identify tumor antigens. Tumor antigens can be identified using multiple methods. One method is comparing proteins, peptides, and mRNA from tumor cells with healthy cells, using proteomics, mass spectrometry and expression profiling. With the gained data possible tumor antigens can be predicted. Another method used is identifying the epitopes which are recognized by tumor-specific antibodies or T cells in the blood of patients. Adapted from (Stevanovic 2002).

expressed in multiple different tumors and silenced in normal cells (Coulie et al. 2001; Lucas et al. 2008).Differentiation antigens are antigens expressed by tumor cells, although these antigens are also found to be expressed by the cells from which the tumor cells are originated. Overexpressed antigens are molecules upregulated in cancers cells compared to normal cells (Coulie et al. 2001).

Tumor antigens might be interesting as molecules mediating the dual reactivity of Vδ2$^-$ γδ T cells, since these antigens are found to be expressed on tumor cells. However, for this dual reactivity also expression of these antigens on CMV-infected cells is crucial. It was found that heat shock proteins and adhesion molecules can be tumor antigens (Wang et al. 2000; Manjili et al. 2004; Siu 2009). The expression of these proteins and some other tumor antigens is described for tumors cells and CMV-infected cells in this chapter.

Heat shock proteins

Heat shock proteins (HSPs) are a family of proteins that possess similar functions and are named according to their molecule weight. All HSPs have a peptide-binding domain that binds hydrophobic residues of substrate proteins and an adenine nucleotide binding domain that binds and hydrolyzes ATP (Hartl 1996). HSPs function as chaperones important in protein folding, transport, and repair (Hartl 1996). HSPs are found to be overexpressed during environmental stress, leading to protection of the cells from damage (Zugel et al. 1999). They are mostly detected in the cytosol, although they are also found on the cell surface of transformed cells (Ferrarini et al. 1992). In many tumors an altered expression of HSPs is described (Morino et al. 1997; Manjili et al. 2004; Tweedle et al. 2010). There are a few tumor-associated heat shock proteins identified, including HSP27, HSP60, and HSP70.

HSP27 participates in multiple processes, like membrane stability, cell migration, cell cycle progression, proinflammatory gene expression, differentiation, and apoptosis (Kostenko et al. 2009). The protein is also found to be overexpressed in many cancer cells, like rectal cancer, breast cancer, and prostate cancer (Morino et al. 1997; Tweedle et al. 2010). In these cells the protein promotes treatment resistance and apoptosis inhibition, via inhibiting the apoptosome and caspace activation complex (Garrido et al. 2003). It has not been established, however, whether the cytomegalovirus alters the expression of HSP27. Interestingly, it is known that other viruses, like EBV and HIV, can upregulate the expression of the HSP27 (Wainberg et al. 1997; Fukagawa et al. 2008). EBV is, like CMV, also a herpesvirus, which could be an indication that CMV might also alter HSP27 expression. However, this feature needs to be established. The study of Mahvi *et al.* demonstrates that upregulation of HSP27 in a breast cancer cell line (MCF-7) leads to increased susceptibility of the cells to γδ T cells, indicating that HSP27 can be an antigen for γδ T cells (Mahvi et al. 1993). This suggest that if CMV does upregulate HSP27 expression, HSP27 could be a shared activating antigen that could plays a role in the dual reactivity of Vδ2$^-$ γδ T cells.

HSP60 is also found to be overexpressed on the cell surface of tumor cells (Thomas et al. 2000; Cappello et al. 2008). It was found to play a role in transport of mitochondrial proteins, replication of mitochondrial DNA, and apoptosis inhibition (Cappello et al. 2008). To mediate the dual reactivity of Vδ2- γδ T cells towards both tumor and CMV-infected cells, the protein must also be expressed on CMV-infected cells. However, no overexpression of HSP60 has been found in these infected cells (Bason et al. 2003). Interestingly, Bason *et al.* found that antibodies against CMV can also induce cytotoxicity and thereby amplify endothelial damage. They showed that these CMV-reactive antibodies enhance the endothelial damage by binding to HSP60 expressed on these cells. This indicates that there could be a cross-reactivity of anti-CMV antibodies with HSP60. Indeed, they established that the amino acid sequence at position 153-163 of HSP60 is homologue to CMV-derived proteins, namely UL122 and US28 (Bason et al. 2003). UL122 was found to regulate viral activation and reactivation from latency and it plays a role in sustaining the CMV infection. This protein is located in the cytosol, however, the presence of this protein on the cell surface has not been established. US28 is a chemokine-receptor-like protein expressed on the cell surface. This protein binds a broad spectrum of chemokines, activating cell-type and ligand-specific signaling pathways. The US28 can bind chemokines from the CC-chemokine family, especially RANTES, MCP-1, MIP-1α and MIP-1β, and with an even higher affinity, a chemokine from the CX_3C-chemokine family, namely Fractalkine (Vomaske et al. 2009). The homology of this viral protein with HSP60 might also promote HSP60-reactive $V\delta2^-$ γδ T cells. There are already HSP60 reactive $V\delta2^-$ γδ T cells described by O'Brien *et al.*, indicating that this protein can be an antigen for these T cells. The overexpression of HSP60 on tumor cells, the cross-reactivity of the anti-CMV antibodies with HSP60, and the fact that $V\delta2^-$ γδ T cellscan be reactive against HSP60 could indicate that the protein might play a role in the dual reactivity of $V\delta2^-$ γδ T cells toward both tumor and CMV-infected cells.

HSP70 has also been found to be overexpressed on several tumors, like oesophageal cancer (Thomas et al. 2000). It plays a role in multiple processes, like cell growth, DNA replication, transcription, protein folding, proteolysis, and tumor immunogenicity. It was found that also during CMV infection, the expression of HSP70 is upregulated (Zhu et al. 1998; Ohgitani et al. 1999). This could indicate that HSP70 could be an antigen for both tumor and CMV-infected cells. It has already been shown by Bonorino *et al.* that γδ T cells can be reactive against HSP70 (Bonorino et al. 1998). However, the study of Thomas *et al.* showed that the majority of the HSP70-reactive γδ T cells were Vγ9Vδ2 T cells, although they also found that $V\delta1^+$ γδ T cells can recognize and lyse HSP70 positive oesophageal tumor cells (Thomas et al. 2000). This indicates that HSP70 can evoke a cytotoxic $V\delta2^-$ γδ T cell response. These results suggest that HSP70 could be an interesting candidate for further research for mediating the reactivity of Vδ2- γδ T cells towards tumor and CMV-infected cells.

Taken together, heat shock proteins, especially HSP60 and HSP70 could be good candidates

as antigen causing the dual reactivity against tumor cells and CMV-infected cells of Vδ2$^-$ γδ T cells. Both are upregulated on tumor cells and there is an overexpression of these molecules, or a homologue, on CMV-infected cells and both molecules are also described to be recognized by Vδ2$^-$ γδ T cells.

Cell adhesion molecules

Cell adhesion molecules (CAMs) can be cell-cell adhesion molecules or cell-extracellular matrix adhesion molecules and with these interactions they can control cellular behavior. There are different types of CAM families, like integrins, cadherins, selectins, mucin-like CAM, CD44 family, and the immunoglobulin (Ig)-superfamily CAM (Figure 7; for review see Lodish et al. 2000). Cadherin and Ig-superfamily form homophillic interactions, meaning that these molecules mediate adhesion between cells of a single cell type. mucin-like CAM, selectins and integrins form heterophilic interactions and thereby mediate adhesion between different cell types (Lodisch et al. 2000).

Integrins mediate cell-cell and cell-substrate interactions and are Ca^{2+}-dependent. They can also generate intracellular signals, leading to cellular growth. Integrins bind to specific proteins, including collagen, RGD (amino acid code for arginine-glycine-aspartic acid found on some proteins) and laminin molecules (Lodish et al. 2000). Cadherins are responsible for Ca^{2+}-dependent cell-cell adhesion and mediating linking of the actin cytoskeleton of these cells. These molecules are transmembrane glycoproteins, which passes the membrane once and which forms dimers or oligomers. Cadherins contain also five or six cadherin repeats related to immunoglobulin. Selectins are carbohydrate-binding proteins, which facilitate Ca^{2+}-dependent cell-cell interactions between immune cells and the endothelial cells. Selectins bind to specific oligosaccharides on glycoproteins and glycolipids. The Ig-superfamiliy CAMs are Ca^{2+}-independent and contain one or more Ig-like domains and are important in cell-cell adhesion (Lodish et al. 2000).

Adhesion molecules have been found to be expressed on tumor cells and are described as tumor antigens. Interesting adhesion molecules are Intercellular adhesion molecule 1 (ICAM-1), CD44, and β1 integrins, since these were found on multiple tumors.

ICAM-1 is a member of the Ig-like CAM and is expressed by several cell types, like endothelial cells and leukocytes (Lawson et al. 2009). ICAM-1 plays an important role in the innate and adaptive immune responses, since the molecule is involved in the transendothelial migration of leukocytes and in the interaction between antigen presenting cells and T cells (Lawson et al. 2009). ICAM-1 can also induce signaltransduction, leading to rearrangement of the actin cytoskeleton which is important for this transendothelial migration (Durieu-Trautmann et al. 1994) ICAM-1 was found to be expressed on several tumors, like melanoma, prostate cancer, and parcreatic cancer (Johnson et al. 1989; Conrad et al. 2009; Roland et al. 2010). High expression of ICAM-1 on tumor cells was found

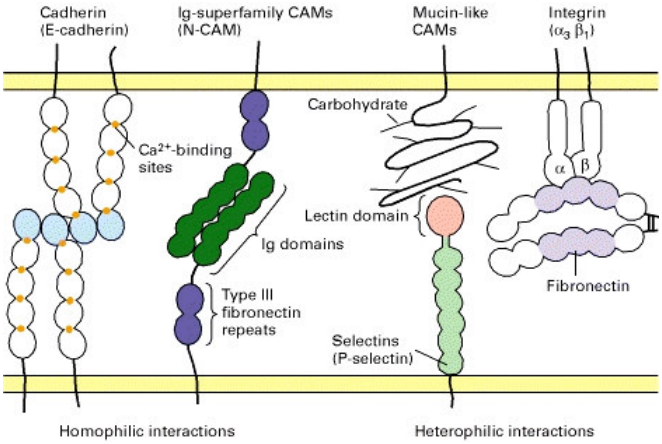

Figure 7 – The five major cell adhesion molecules families. Cadherin and Immunoglobulin-superfamily cell adhesion molecules form homophillic interactions. Mucin-like cell adhesion molecules, selectins and integrins form heterophillic interactions (Lodish et al. 2000).

to be associated with the progression of tumors (Johnson et al. 1989; Roland et al. 2010). The expression of ICAM-1 is also influenced by the infection of CMV. This virus causes overexpression of the adhesion molecule (Ito et al. 1995a; Ito et al. 1995b; Larcher et al. 1997; Helantera et al. 2005). This suggests that both cellular transformation and CMV infection causes a higher expression of ICAM-1. It was also found that the degree of surface expression of ICAM-1 is correlated with the degree of lysis of the cells by γδ T cells. The study of Lui *et al.* determined that the amount of expression of ICAM-1 molecules on pancreatic cancer cells influences the binding to and killing of the cancer cells by γδ T cells. Pancreatic cancer cells, which did not express ICAM-1, were resistant to γδ T cells. Transfection of these cells with ICAM-1, however, made these cells more susceptible for γδ T cell binding and killing (Liu et al. 2009). This indicates that ICAM-1 may play an important role in the reactivity of γδ T cell towards these pancreatic cancer cells. This beneficial effect of ICAM-1 on the lysis of ICAM-1-positive tumor cells could indicate that ICAM-1 might be an antigen for the γδ T cells. However, another explanation might be that ICAM-1 promotes the binding of the γδ T cells to their target cells, enable them lyse the target cells. Nevertheless, ICAM-1 could play a role in the dual reactivity of $V\delta2^-$ γδ T cells.

CD44 is a transmembrane glycoproteins and forms another CAM family. All family members have a large extracellular domain, a transmembrane domain, and a cytoplasmic domain. Through insertion of variable exons via alternative splicing, the CD44 family members have variations in the extracellular domain (Orian-Rousseau 2010). There are 10 variable exons (v1-v10) which are located close to the transmembrane region and which give rise to 10 isoforms (Figure 8). The most common CD44 molecule, without this variable exon, is called the smallest isoform (CD44s) (Figure 8). The extracellular domain contains a hyaluronic acid (HA) binding domain, which is the main ligand for CD44 (Orian-Rousseau 2010). CD44 can, however, also interact with other molecules, like collagen, fibronectin, and laminin (Lesley et al. 1993). CD44 molecules can regulate growth, survival,

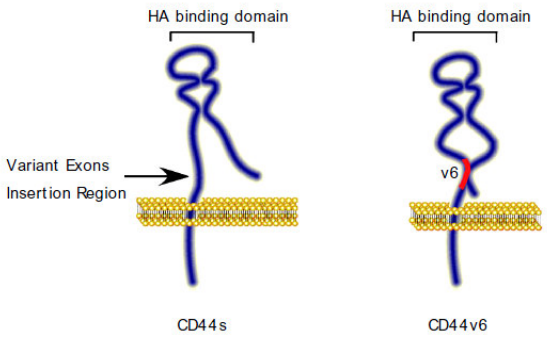

Figure 8 – Isoforms of CD44. The CD44 family has different isoforms, formed by the insert of the variant regions. CD44s is the most common isoform, which has not a variable region. HA: hyaluronic acid (Orian-Rousseau 2010).

differentiation, and migration of cells, which could be contributing to tumor progression (Hamann et al. 1995; Charrad et al. 1999; Allouche et al. 2000). CD44 overexpression was found on different types of tumors, like a pancreatic carcinoma, gallbladder carcinoma, lung cancer, and endometrial cancers (Katsura et al. 1998; Mizera-Nyczak et al. 2001; Yanagisawa et al. 2001). The expression of CD44 has been found to be correlated with aggressive stages of various cancers (Orian-Rousseau 2010). Moreover, blockade of this molecule has been shown to decrease tumor growth and metastasis in different tumors, like melanoma and lymphoma (Sy et al. 1992; Guo et al. 1994). The expression of CD44 is not only influenced by cellular transformation, also CMV can alter the expression of this molecule. The study of Ito *et al*. shows that CMV-infected human embryonic fibroblasts have a higher expression of CD44 (Ito et al. 1995a). They stated that the overexpression of this molecule might contribute to the CMV-induced inflammation. The overexpression of CD44 on both tumor cells and CMV-infected cells suggest that this molecule could mediate the $V\delta2^-$ γδ T cell dual reactivity towards both cell types.

β1 integrin is a subunit of a heterodimeric transmembrane receptor. Integrins consist of an α and a β subunit. There are 18 different α subunits and 8 different β subunits, which together can form 24 integrin receptors (Brakebusch et al. 2002). There are different subfamilies of integrins, distinguished according to their ligand specificity, they can form collagen receptors, laminin receptors, and RGD receptors. The β1 integrin subunit can pair with most of the α subunits and is thereby present in most of the integrin receptors. When integrins bind to their ligands the cytoplasmic domain connects to the actin cytoskeleton via integrin associated molecules, which elicits intracellular signalling. On various tumor types, this molecule has been found to be upregulated. For example, after analysis of the surface molecules expression of prostate cancer cells, overexpression of the β1 integrin was found (Rentala et al. 2010). Also on primary human breast carcinomas, a higher expression of the β1 integrin (associated with the α3 subunits) was found. Moreover, on the metastases of these primary tumors there was an overexpression found of this molecule (Morini et al. 2000). CMV has also been found to influence the expression of this adhesion

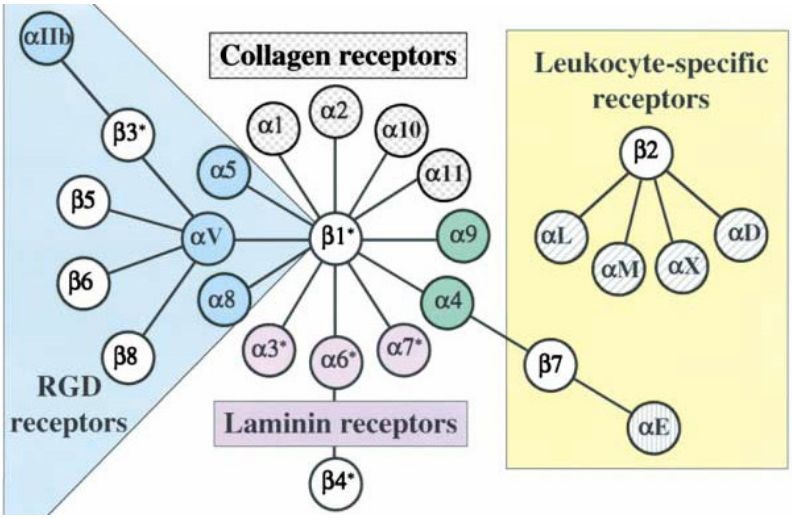

Figure 9 – Integrin receptor family. Each integrin is a dimer consisting of an α and a β subunits. This figure shows the combinations of the integrin receptors. The different subfamilies are defined with different colours. α subunits can be specific for laminin (purple), collagen (gray stippling) or RGD (blue). The subunits in the yellow box are restricted on leukocytes. The green subunits are restricted to chordates (Hynes 2002).

molecule. The study of Ito *et al.* shows an increase in β1 integrin expression on CMV-infected human embryonic fibroblasts (Ito et al. 1995a). Shahgasempour and colleagues also show an upregulation of integrins with the β1 subunit (α6β1 and α3β1) in CMV-infected human umbilical vein endothelial cells, however they also demonstrate that other integrins with the β1 subunit were downregulated by the virus (α5β1 and α2β1) (Shahgasempour et al. 1997). The upregulation of the β1 integrin subunit on both tumor and CMV-infected cells suggests that also this cell adhesion molecule could be a antigen that contributes to the dual reactivity of Vδ2$^-$ γδ T cells.

Taken together, CAMs, like ICAM-1, CD44, and β1 integrin, could be candidates as shared antigens for tumors and CMV infection mediating the dual reactivity of Vδ2- γδ T cells , since these molecules are overexpressed on both cell types. On activated endothelial cells, however, also upregulation of CAMs, like ICAM-1, have been found (Tsoyi et al. 2010). This might suggest that CAMs are not good candidates as shared activation antigens. However, like αβ T cells, γδ T cells probably also need co-stimulation and adhesion-facilitating molecules. This suggests that CAMs might act as co-stimulator or might need other molecules for co-stimulation or that CAMS facilitate the adhesion of γδ T cells. This could explain why γδ T cells are not reactive against activated endothelial cells. Therefore, CAMs may play a role in mediating the reactivity against tumor cells and CMV-infection by Vδ2$^-$ γδ T cells.

Other tumor antigens

Besides heat shock proteins and adhesion molecules, many other tumor antigens have been identified and some of these antigens may also be antigen candidates mediating the dual reactivity of Vδ2$^-$ γδ T cells. For example, interferon-induced transmembrane protein 1 (IFITM1) could be an interesting tumor antigen. IFITM1, also called 9-27 or Leu13, is a member of the IFN-inducible

transmembrane protein family and the transcription of this protein can be induced by IFN-α and IFN-γ (Yu et al. 2010). It is part of a membrane complex that provides antiproliferative and adhesion signals in lymphocytes (Sato et al. 1997). IFITM1 has an elevated expression in different cancer types, like gastric cancer, head and neck cancer, and glioma cell lines and is associated with the progression in these cancers (Yang et al. 2005; Hatano et al. 2008; Yu et al. 2010). The protein might contribute to the progression of cancer by enhancing the cell migration and the invasive capacity (Yang et al. 2005; Hatano et al. 2008; Yu et al. 2010). To be a candidate as shared antigen for both tumor and CMV-infected cells, this protein must also be expressed on the surface of CMV infected cells. Unfortunately, the surface expression of IFITM1 has not been established. However, some studies have shown that the mRNA expression of this protein is altered (Crowe et al. 2004; Mezger et al. 2009). For example, Mezger *et al.* found that in CMV-infected dentritic cells the mRNA level of IFITM1 is elevated (Mezger et al. 2009). Elevated levels of mRNA might not directly mean that also the protein expression is increased, though it might be a first indication that the protein expression could also be altered. When IFITM1 protein is also overexpressed by CMV-infected cells, like on tumor cells, this protein could be an interesting candidate as shared activating antigen for cancer and CMV infection. IFITM1, however, might also play a role as an adhesion-facilitating molecule, since this molecule has been found to provide adhesion signals in lymphocytes (Sato et al. 1997). The theory that IFITM1 could mediate the dual reactivity is supported by the fact that leukemias expressing IFITM1 are more susceptible for γδ T cells cytotoxicity (Gomes et al. 2010).

Another tumor antigen is CD36, which is a membrane glycoprotein, containing two transmembrane domains, two short cytoplasmic domains and a large extracellular domain (Gruarin et al. 2000). This receptor is present on many different cell types, like platelets, mononuclear phagocytes, adipocytes, hepatocytes, myocytes, and some epithelia. The ligands for this receptor are thrombospondin-1 and other proteins with thrombospondin type I repeats (Asch et al. 1987). CD36 can inhibit angiogenesis by inhibiting pro-angiogenic growth factor, although it can also acts as a scavenger receptor recognized by cells of the innate immune system (Endemann et al. 1993). This molecule was found to be expressed on tumor cells. For example, a high expression of this molecule found on neoplastic B-cells from patients with CLL (Rutella et al. 1999). Also expression of CD36 was found on epidermal cell-derived tumors (Allen et al. 1991). Besides cellular transformation, also CMV can influence the expression of CD36. The study of Carlquist *et al.* demonstrates that CMV infection causes CD36 mRNA accumulation and increased cell surface expression (Carlquist et al. 2004). The findings that both tumor and CMV-infected cells express CD36 could suggest that this receptor might be a shared antigen. Unfortunately, as stated above, this molecule is expressed on a variety of healthy cells, indicating that CD36 might not be a principal candidate as antigen causing the dual reactivity of $V\delta2^-$ γδ T cells.

Discussion

Unfortunately, there is still a lot unknown about the mechanism of γδ T cells, despite the recent research. These cells, however, have been shown to be involved in many pathological contexts and were found to play a role in the defense against certain viruses and a variety of tumor cells (Sindhu et al. 2003; Corvaisier et al. 2005; Kabelitz et al. 2007; Couzi et al. 2010). It was recently shown that Vδ2$^-$ γδ T cells can be dual reactive against both tumor cells and CMV-infected cells (Halary et al. 2005). This dual reactivity could be important for patients receiving an allo-SCT, since these patients are more susceptible for CMV infection (Hebart et al. 2004). This infection is one of the causes of morbidity and mortality in these patients, meaning that prevention of this infection or reactivation could be beneficial for their prognosis (Hebart et al. 2004). The dual reactive Vδ2$^-$ γδ T cells could be interesting as a treatment, since these cells are known to kill tumor cells, while at the same time control CMV infection and reactivation. The reactivity towards both tumor and CMV-infected cells is most likely caused by antigens expressed on both cell types. There are, however, only a few antigens identified for γδ T cells and these are mainly recognized by Vδ2$^+$ γδ T cells (Kabelitz et al. 2007). This review presents possible antigens for Vδ2$^-$ γδ T cells shared by transformed cells and CMV-infected cells, which might underlie the dual reactivity of these T cells.

To identify possible candidate molecules, a part of this review is focused on the MHC superfamily. All investigated members of this superfamily were found expressed on the cell surface of tumor cells, although unfortunately, CMV can manipulate the expression of these proteins. Viral protein UL16, for example, can sequestrate multiple members of this superfamily, including MICB, ULBP1, ULBP2, and ULBP6, in the ER or Golgi apparatus (Odeberg et al. 2003; Collins 2004; Wilkinson et al. 2008). There are, however, still proteins in this family that might be possible candidates. For example, EPCR, a member of this superfamily, was still found expressed on the cell surface of CMV-infected cells, indicating that this protein might mediate the dual reactivity of Vδ2$^-$ γδ T cells. Also a variant of MICA, MICA*008, which is another member of the MHC superfamily and a known ligand of Vδ2$^-$ γδ T cells, might mediate this dual reactivity (Ashiru et al. 2009; Ashiru et al. 2010). This truncated variant is not downregulated by CMV and is still expressed on the cell surface, making it a possible antigen for Vδ2$^-$ γδ T cells. Unfortunately, this protein was also found expressed on the surface of exosomes secreted by tumor cells, which could be a mechanism to evade the immune system (Ashiru et al. 2010). Taken together, both EPCR and MICA*008 might be interesting for further research to establish their role in the reactivity of Vδ2$^-$ γδ T cells towards both tumor and CMV-infected cells.

Besides the MHC superfamily, this review also focused on tumor antigen as possible candidates as shared antigens for Vδ2$^-$ γδ T cells. A variety of tumor antigens were also found to be

expressed by cells infected with CMV. Antigens on which this review is focused consist mainly of heat shock proteins and adhesion molecules. Heat shock proteins are interesting for further research for their role in the dual reactivity of Vδ2$^-$ γδ T cells, since these proteins are expressed by stressed cell and γδ T cells are known to recognize stress-related antigens (Kabelitz et al. 2007). Especially, HSP27, HSP60, and HSP70 are interesting as possible shared antigens, since these proteins are known tumor-associated antigens and are found to be overexpressed on multiple tumors (Morino et al. 1997; Thomas et al. 2000; Cappello et al. 2008). The effect of CMV-infection on HSP27 has not been established, however, another herpes virus (EBV) has been shown to upregulate HSP27, which may suggest that also CMV might influence the expression of HSP27 (Fukagawa et al. 2008). Furthermore, no upregulation of HSP60 has been established, however, it was found that CMV-reactive antibodies are also reactive against HSP60. This was caused by homology between HSP60 and some viral proteins (Bason et al. 2003). The cell surface expression of HSP70 has been shown to be upregulated by CMV (Zhu et al. 1998). These findings indicate that both HSP60 and HSP70 could mediate the dual reactivity of Vδ2$^-$ γδ T cells. Adhesion molecules might also contribute to the dual reactivity of Vδ2$^-$ γδ T cells by facilitating the adhesion of the T cells to the target cells or as co-stimulatory molecules. Some of these molecules, like ICAM-1, CD44, and β1 integrins, are found to be overexpressed on tumor cells (Johnson et al. 1989; Morini et al. 2000; Yanagisawa et al. 2001). These three types of adhesion molecules were also found overexpressed on CMV-infected cells, making these proteins potential candidates as antigens for the dual reactive Vδ2$^-$ γδ T cells (Ito et al. 1995a; Ito et al. 1995b; Helantera et al. 2005). Besides heat shock proteins and adhesion molecules also other tumor antigens, like IFITM1, could be shared antigens for Vδ2$^-$ γδ T cells. The protein expression of IFITM1 has not been established on CMV-infected cells, although the mRNA expression was found to be upregulated after infection. Taken together, heat shock proteins HSP60 and HSP70, adhesion molecules ICAM-1, CD44, and β1 integrins and tumor antigen IFITM1 might be candidates for the reactivity of Vδ2$^-$ γδ T cells towards both tumor and CMV-infected cells and could thereby be interesting for further research to establish their role in this dual reactivity.

Another interesting area in which possible shared antigens might be found and which is not included in this review, could be shared subcellular events after cell transformation and CMV infection. A remarkable feature after CMV infection is that the virus influences the lipid metabolism of the cells (Sanchez et al. 2010). The virus causes, for example, an intracellular accumulation of cholesterol and *de novo* synthesis of sphinholipids (Abrahamsen et al. 1996; Machesky et al. 2008). The study of Sanchez and colleagues supports the theory that lipid metabolism is altered during CMV infection by showing that the expression of the ATP-binding cassette transporter A1, which causes efflux of cholesterol from the cells, is decreased (Sanchez et al. 2010). This causes accumulation of lipids in the infected cells and may lead to higher lipid antigen presentation on CD1 molecules.

Interestingly, these molecules are known to be recognized by γδ T cells. Although unfortunately, CD1 expression on the cell surface is downregulated by CMV, making the theory that lipid accumulation could be a provider of shared antigens less plausible (Hertel et al. 2003). However, it may be interesting to investigate for other similarities in subcellular events in tumor cells and CMV-infected cells, which might lead to identification of shared antigens for dual reactive Vδ2$^-$ γδ T cells.

In this review I tried to establish candidates for shared antigens expressed on tumor and CMV-infected cells. Identifying antigens of dual reactive γδ T cells will provide us a better understanding of the pathophysiological function of γδ T cells *in* vivo, as well as the molecular mechanisms of γδ T cell activation. However, for the application of such γδ TCRs in the clinic, several problems need to be tackled in the near future. For example, the expression of antigens are very divers on different types of cancers, meaning that found candidates might be expressed only on a few types of cancers, making the Vδ2$^-$ γδ T cells reactive for these antigens less usable for a wide population (Lucas et al. 2008). Moreover, the activation of Vδ2$^-$ γδ T cells resembles most likely the activation of αβ T cells, meaning that co-stimulatory molecules may be necessary (McAdam et al. 1998). This suggests that one molecule is not enough to activate the Vδ2$^-$ γδ T cells, meaning that multiple molecules expressed on both tumor and CMV-infected cells might be necessary. The found candidate molecules, however, could be the first step in understanding the function of Vδ2$^-$ γδ T cells as well as the mechanism of Vδ2$^-$ γδ T cells activation.

In summary, this review describes possible candidates as shared antigens for both tumor and CMV-infected cells which may mediate the dual reactivity of Vδ2$^-$ γδ T cells towards these cells. Most of these candidates were known tumor antigens, like heat shock proteins (HSP60 and HSP70), adhesion molecules (ICAM-1, CD44, and β1 integrins), and IFITM1. However, besides tumor antigens, also some members of the MHC superfamily are considered to be possible antigens underlying the dual reactivity, namely MICA*008 and EPCR. Further research should establish the role of these molecules in the dual reactivity of Vδ2$^-$ γδ T cells towards cancer and CMV infection.

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References

Abrahamsen, L. H., Clay, M. J., Lyle, J. M., Zink, J. M., Fredrikson, L. J., DeSiervo, A. J. and Jerkofsky, M. A. (1996). "The effects of cytomegalovirus infection on polar lipids and neutral lipids in cultured human cells." Intervirology **39**(4): 223-229.

Adly, M. A., Assaf, H. A. and Hussein, M. (2005). "Expression of CD1d in human scalp skin and hair follicles: hair cycle related alterations." J Clin Pathol **58**(12): 1278-1282.

Agrati, C., D'Offizi, G., Narciso, P., Abrignani, S., Ippolito, G., Colizzi, V. and Poccia, F. (2001). "Vdelta1 T lymphocytes expressing a Th1 phenotype are the major gammadelta T cell subset infiltrating the liver of HCV-infected persons." Mol Med **7**(1): 11-19.

Allen, M. H., Barker, J. N. and MacDonald, D. M. (1991). "Keratinocyte expression of CD36 antigen in benign and malignant epidermal cell-derived tumours." J Cutan Pathol **18**(3): 198-203.

Allouche, M., Charrad, R. S., Bettaieb, A., Greenland, C., Grignon, C. and Smadja-Joffe, F. (2000). "Ligation of the CD44 adhesion molecule inhibits drug-induced apoptosis in human myeloid leukemia cells." Blood **96**(3): 1187-1190.

Asch, A. S., Barnwell, J., Silverstein, R. L. and Nachman, R. L. (1987). "Isolation of the thrombospondin membrane receptor." J Clin Invest **79**(4): 1054-1061.

Ashiru, O., Bennett, N. J., Boyle, L. H., Thomas, M., Trowsdale, J. and Wills, M. R. (2009). "NKG2D ligand MICA is retained in the cis-Golgi apparatus by human cytomegalovirus protein UL142." J Virol **83**(23): 12345-12354.

Ashiru, O., Boutet, P., Fernandez-Messina, L., Aguera-Gonzalez, S., Skepper, J. N., Vales-Gomez, M. and Reyburn, H. T. (2010). "Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes." Cancer Res **70**(2): 481-489.

Bacon, L., Eagle, R. A., Meyer, M., Easom, N., Young, N. T. and Trowsdale, J. (2004). "Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D." J Immunol **173**(2): 1078-1084.

Bason, C., Corrocher, R., Lunardi, C., Puccetti, P., Olivieri, O., Girelli, D., Navone, R., Beri, R., Millo, E., Margonato, A., Martinelli, N. and Puccetti, A. (2003). "Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis." Lancet **362**(9400): 1971-1977.

Ben-Arieh, S. V., Zimerman, B., Smorodinsky, N. I., Yaacubovicz, M., Schechter, C., Bacik, I., Gibbs, J., Bennink, J. R., Yewdell, J. W., Coligan, J. E., Firat, H., Lemonnier, F. and Ehrlich, R. (2001). "Human cytomegalovirus protein US2 interferes with the expression of human HFE, a nonclassical class I major histocompatibility complex molecule that regulates iron homeostasis." J Virol **75**(21): 10557-10562.

Bennett, N. J., Ashiru, O., Morgan, F. J., Pang, Y., Okecha, G., Eagle, R. A., Trowsdale, J., Sissons, J. G. and Wills, M. R. (2010). "Intracellular sequestration of the NKG2D ligand ULBP3 by human cytomegalovirus." J Immunol **185**(2): 1093-1102.

Bialasiewicz, A. A., Ma, J. X. and Richard, G. (1999). "Alpha/beta- and gamma/delta TCR(+) lymphocyte infiltration in necrotising choroidal melanomas." Br J Ophthalmol **83**(9): 1069-1073.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. and Wiley, D. C. (1987). "The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens." Nature **329**(6139): 512-518.

Blumberg, R. S. (1998). "Current concepts in mucosal immunity. II. One size fits all: nonclassical MHC molecules fulfill multiple roles in epithelial cell function." Am J Physiol **274**(2 Pt 1): G227-231.

Boissel, N., Rea, D., Tieng, V., Dulphy, N., Brun, M., Cayuela, J. M., Rousselot, P., Tamouza, R., Le Bouteiller, P., Mahon, F. X., Steinle, A., Charron, D., Dombret, H. and Toubert, A. (2006). "BCR/ABL oncogene directly controls MHC class I chain-related molecule A expression in chronic myelogenous leukemia." J Immunol **176**(8): 5108-5116.

Bonish, B., Jullien, D., Dutronc, Y., Huang, B. B., Modlin, R., Spada, F. M., Porcelli, S. A. and Nickoloff, B. J. (2000). "Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells." J Immunol **165**(7): 4076-4085.

Bonneville, M., O'Brien, R. L. and Born, W. K. (2010). "Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity." Nat Rev Immunol **10**(7): 467-478.

Bonorino, C., Nardi, N. B., Zhang, X. and Wysocki, L. J. (1998). "Characteristics of the strong antibody response to mycobacterial Hsp70: a primary, T cell-dependent IgG response with no evidence of natural priming or gamma delta T cell involvement." J Immunol **161**(10): 5210-5216.

Brakebusch, C., Bouvard, D., Stanchi, F., Sakai, T. and Fassler, R. (2002). "Integrins in invasive growth." J Clin Invest **109**(8): 999-1006.

Brandes, M., Willimann, K. and Moser, B. (2005). "Professional antigen-presentation function by human gammadelta T Cells." Science **309**(5732): 264-268.

Braud, V. M., Allan, D. S. and McMichael, A. J. (1999). "Functions of nonclassical MHC and non-MHC-encoded class I molecules." Curr Opin Immunol **11**(1): 100-108.

Brutkiewicz, R. R. (2006). "CD1d ligands: the good, the bad, and the ugly." J Immunol **177**(2): 769-775.

Cao, W. and He, W. (2004). "UL16 binding proteins." Immunobiology **209**(3): 283-290.

Cappello, F., Conway de Macario, E., Marasa, L., Zummo, G. and Macario, A. J. (2008). "Hsp60 expression, new locations, functions and perspectives for cancer diagnosis and therapy." Cancer Biol Ther **7**(6): 801-809.

Carding, S. R. and Egan, P. J. (2002). "Gammadelta T cells: functional plasticity and heterogeneity." Nat Rev Immunol **2**(5): 336-345.

Carlquist, J. F., Muhlestein, J. B., Horne, B. D., Hart, N. I., Lim, T., Habashi, J., Anderson, J. G. and Anderson, J. L. (2004). "Cytomegalovirus stimulated mRNA accumulation and cell surface expression of the oxidized LDL scavenger receptor, CD36." Atherosclerosis **177**(1): 53-59.

Centelles, M. N., Puy, C., Lopez-Sagaseta, J., Fukudome, K., Montes, R. and Hermida, J. (2010). "Blocking endothelial protein C receptor (EPCR) accelerates thrombus development in vivo." Thromb Haemost **103**(6): 1239-1244.

Chalupny, N. J., Rein-Weston, A., Dosch, S. and Cosman, D. (2006). "Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142." Biochem Biophys Res Commun **346**(1): 175-181.

Charrad, R. S., Li, Y., Delpech, B., Balitrand, N., Clay, D., Jasmin, C., Chomienne, C. and Smadja-Joffe, F. (1999). "Ligation of the CD44 adhesion molecule reverses blockage of differentiation in human acute myeloid leukemia." Nat Med **5**(6): 669-676.

Chen, Y., Cheng, M. and Tian, Z. (2006). "Hepatitis B virus down-regulates expressions of MHC class I molecules on hepatoplastoma cell line." Cell Mol Immunol **3**(5): 373-378.

Choudhary, A., Davodeau, F., Moreau, A., Peyrat, M. A., Bonneville, M. and Jotereau, F. (1995). "Selective lysis of autologous tumor cells by recurrent gamma delta tumor-infiltrating lymphocytes from renal carcinoma." J Immunol **154**(8): 3932-3940.

Collins, R. W. (2004). "Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation." Eur J Immunogenet **31**(3): 105-114.

Conrad, F., Zhu, X., Zhang, X., Chalkley, R. J., Burlingame, A. L., Marks, J. D. and Liu, B. (2009). "Human antibodies targeting cell surface antigens overexpressed by the hormone refractory metastatic prostate cancer cells: ICAM-1 is a tumor antigen that mediates prostate cancer cell invasion." J Mol Med **87**(5): 507-514.

Corvaisier, M., Moreau-Aubry, A., Diez, E., Bennouna, J., Mosnier, J. F., Scotet, E., Bonneville, M. and Jotereau, F. (2005). "V gamma 9V delta 2 T cell response to colon carcinoma cells." J Immunol **175**(8): 5481-5488.

Coulie, P. G., Hanagiri, T. and Takenoyama, M. (2001). "From tumor antigens to immunotherapy." Int J Clin Oncol **6**(4): 163-170.

Couzi, L., Levaillant, Y., Jamai, A., Pitard, V., Lassalle, R., Martin, K., Garrigue, I., Hawchar, O., Siberchicot, F., Moore, N., Moreau, J. F., Dechanet-Merville, J. and Merville, P. (2010). "Cytomegalovirus-induced gammadelta T cells associate with reduced cancer risk after kidney transplantation." J Am Soc Nephrol **21**(1): 181-188.

Crowe, W. E., Maglova, L. M., Ponka, P. and Russell, J. M. (2004). "Human cytomegalovirus-induced host cell enlargement is iron dependent." Am J Physiol Cell Physiol **287**(4): C1023-1030.

De Maria, A., Ferrazin, A., Ferrini, S., Ciccone, E., Terragna, A. and Moretta, L. (1992). "Selective increase of a subset of T cell receptor gamma delta T lymphocytes in the peripheral blood of patients with human immunodeficiency virus type 1 infection." J Infect Dis **165**(5): 917-919.

De Rosa, S. C., Mitra, D. K., Watanabe, N., Herzenberg, L. A. and Roederer, M. (2001). "Vdelta1 and Vdelta2 gammadelta T cells express distinct surface markers and might be developmentally distinct lineages." J Leukoc Biol **70**(4): 518-526.

Dechanet, J., Merville, P., Lim, A., Retiere, C., Pitard, V., Lafarge, X., Michelson, S., Meric, C., Hallet, M. M., Kourilsky, P., Potaux, L., Bonneville, M. and Moreau, J. F. (1999). "Implication of gammadelta T cells in the human immune response to cytomegalovirus." J Clin Invest **103**(10): 1437-1449.

Deusch, K., Luling, F., Reich, K., Classen, M., Wagner, H. and Pfeffer, K. (1991). "A major fraction of human intraepithelial lymphocytes simultaneously expresses the gamma/delta T cell receptor, the CD8 accessory molecule and preferentially uses the V delta 1 gene segment." Eur J Immunol **21**(4): 1053-1059.

Devilder, M. C., Maillet, S., Bouyge-Moreau, I., Donnadieu, E., Bonneville, M. and Scotet, E. (2006). "Potentiation of antigen-stimulated V gamma 9V delta 2 T cell cytokine production by immature dendritic cells (DC) and reciprocal effect on DC maturation." J Immunol **176**(3): 1386-1393.

Dranoff, G. (2004). "Cytokines in cancer pathogenesis and cancer therapy." Nat Rev Cancer **4**(1): 11-22.

Duprat, E., Lefranc, M. P. and Gascuel, O. (2006). "A simple method to predict protein-binding from aligned sequences--application to MHC superfamily and beta2-microglobulin." Bioinformatics **22**(4): 453-459.

Durieu-Trautmann, O., Chaverot, N., Cazaubon, S., Strosberg, A. D. and Couraud, P. O. (1994). "Intercellular adhesion molecule 1 activation induces tyrosine phosphorylation of the cytoskeleton-associated protein cortactin in brain microvessel endothelial cells." J Biol Chem **269**(17): 12536-12540.

Eagle, R. A., Traherne, J. A., Ashiru, O., Wills, M. R. and Trowsdale, J. (2006). "Regulation of NKG2D ligand gene expression." Hum Immunol **67**(3): 159-169.

Eagle, R. A. and Trowsdale, J. (2007). "Promiscuity and the single receptor: NKG2D." Nat Rev Immunol **7**(9): 737-744.

Eagle, R. A., Jafferji, I. and Barrow, A. D. (2009a). "Beyond Stressed Self: Evidence for NKG2D Ligand Expression on Healthy Cells." Curr Immunol Rev **5**(1): 22-34.

Eagle, R. A., Traherne, J. A., Hair, J. R., Jafferji, I. and Trowsdale, J. (2009b). "ULBP6/RAET1L is an additional human NKG2D ligand." Eur J Immunol **39**(11): 3207-3216.

Endemann, G., Stanton, L. W., Madden, K. S., Bryant, C. M., White, R. T. and Protter, A. A. (1993). "CD36 is a receptor for oxidized low density lipoprotein." J Biol Chem **268**(16): 11811-11816.

Exley, M., Garcia, J., Wilson, S. B., Spada, F., Gerdes, D., Tahir, S. M., Patton, K. T., Blumberg, R. S., Porcelli, S., Chott, A. and Balk, S. P. (2000). "CD1d structure and regulation on human thymocytes, peripheral blood T cells, B cells and monocytes." Immunology **100**(1): 37-47.

Fais, F., Morabito, F., Stelitano, C., Callea, V., Zanardi, S., Scudeletti, M., Varese, P., Ciccone, E. and Grossi, C. E. (2004). "CD1d is expressed on B-chronic lymphocytic leukemia cells and mediates alpha-galactosylceramide presentation to natural killer T lymphocytes." Int J Cancer **109**(3): 402-411.

Ferrarini, M., Heltai, S., Zocchi, M. R. and Rugarli, C. (1992). "Unusual expression and localization of heat-shock proteins in human tumor cells." Int J Cancer **51**(4): 613-619.

Fukagawa, Y., Nishikawa, J., Kuramitsu, Y., Iwakiri, D., Takada, K., Imai, S., Satake, M., Okamoto, T., Fujimoto, M., Okita, K., Nakamura, K. and Sakaida, I. (2008). "Epstein-Barr virus upregulates phosphorylated heat shock protein 27 kDa in carcinoma cells using the phosphoinositide 3-kinase/Akt pathway." Electrophoresis **29**(15): 3192-3200.

Fukudome, K. and Esmon, C. T. (1994). "Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor." J Biol Chem **269**(42): 26486-26491.

Garrido, C., Schmitt, E., Cande, C., Vahsen, N., Parcellier, A. and Kroemer, G. (2003). "HSP27 and HSP70: potentially oncogenic apoptosis inhibitors." Cell Cycle **2**(6): 579-584.

Gomes, A. Q., Correia, D. V., Grosso, A. R., Lanca, T., Ferreira, C., Lacerda, J. F., Barata, J. T., Silva, M. G. and Silva-Santos, B. (2010). "Identification of a panel of ten cell surface protein antigens associated with immunotargeting of leukemias and lymphomas by peripheral blood gammadelta T cells." Haematologica **95**(8): 1397-1404.

Groh, V., Steinle, A., Bauer, S. and Spies, T. (1998). "Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells." Science **279**(5357): 1737-1740.

Groh, V., Rhinehart, R., Secrist, H., Bauer, S., Grabstein, K. H. and Spies, T. (1999). "Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB." Proc Natl Acad Sci U S A **96**(12): 6879-6884.

Gruarin, P., Thorne, R. F., Dorahy, D. J., Burns, G. F., Sitia, R. and Alessio, M. (2000). "CD36 is a ditopic glycoprotein with the N-terminal domain implicated in intracellular transport." Biochem Biophys Res Commun **275**(2): 446-454.

Guo, Y., Ma, J., Wang, J., Che, X., Narula, J., Bigby, M., Wu, M. and Sy, M. S. (1994). "Inhibition of human melanoma growth and metastasis in vivo by anti-CD44 monoclonal antibody." Cancer Res **54**(6): 1561-1565.

Halary, F., Pitard, V., Dlubek, D., Krzysiek, R., de la Salle, H., Merville, P., Dromer, C., Emilie, D., Moreau, J. F. and Dechanet-Merville, J. (2005). "Shared reactivity of V{delta}2(neg) {gamma}{delta} T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells." J Exp Med **201**(10): 1567-1578.

Hamann, K. J., Dowling, T. L., Neeley, S. P., Grant, J. A. and Leff, A. R. (1995). "Hyaluronic acid enhances cell proliferation during eosinopoiesis through the CD44 surface antigen." J Immunol **154**(8): 4073-4080.

Hanninen, M. M., Haapasalo, J., Haapasalo, H., Fleming, R. E., Britton, R. S., Bacon, B. R. and Parkkila, S. (2009). "Expression of iron-related genes in human brain and brain tumors." BMC Neurosci **10**: 36.

Hartl, F. U. (1996). "Molecular chaperones in cellular protein folding." Nature **381**(6583): 571-579.

Hatano, H., Kudo, Y., Ogawa, I., Tsunematsu, T., Kikuchi, A., Abiko, Y. and Takata, T. (2008). "IFN-induced transmembrane protein 1 promotes invasion at early stage of head and neck cancer progression." Clin Cancer Res **14**(19): 6097-6105.

Hebart, H. and Einsele, H. (2004). "Clinical aspects of CMV infection after stem cell transplantation." Hum Immunol **65**(5): 432-436.

Helantera, I., Loginov, R., Koskinen, P., Tornroth, T., Gronhagen-Riska, C. and Lautenschlager, I. (2005). "Persistent cytomegalovirus infection is associated with increased expression of TGF-beta1, PDGF-AA and ICAM-1 and arterial intimal thickening in kidney allografts." Nephrol Dial Transplant **20**(4): 790-796.

Hertel, L., Lacaille, V. G., Strobl, H., Mellins, E. D. and Mocarski, E. S. (2003). "Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus." J Virol **77**(13): 7563-7574.

Hughes, A. L. and Nei, M. (1989). "Evolution of the major histocompatibility complex: independent origin of nonclassical class I genes in different groups of mammals." Mol Biol Evol **6**(6): 559-579.

Hynes, R. O. (2002). "Integrins: bidirectional, allosteric signaling machines." Cell **110**(6): 673-687.

Ito, M., Watanabe, M., Ihara, T., Kamiya, H. and Sakurai, M. (1995a). "Increased expression of adhesion molecules (CD54, CD29 and CD44) on fibroblasts infected with cytomegalovirus." Microbiol Immunol **39**(2): 129-133.

Ito, M., Watanabe, M., Kamiya, H. and Sakurai, M. (1995b). "Changes of adhesion molecule (LFA-1, ICAM-1) expression on memory T cells activated with cytomegalovirus antigen." Cell Immunol **160**(1): 8-13.

Johnson, J. P., Stade, B. G., Holzmann, B., Schwable, W. and Riethmuller, G. (1989). "De novo expression of intercellular-adhesion molecule 1 in melanoma correlates with increased risk of metastasis." Proc Natl Acad Sci U S A **86**(2): 641-644.

Kabelitz, D., Wesch, D. and He, W. (2007). "Perspectives of gammadelta T cells in tumor immunology." Cancer Res **67**(1): 5-8.

Kato, N., Tanaka, J., Sugita, J., Toubai, T., Miura, Y., Ibata, M., Syono, Y., Ota, S., Kondo, T., Asaka, M. and Imamura, M. (2007). "Regulation of the expression of MHC class I-related chain A, B (MICA, MICB) via chromatin remodeling and its impact on the susceptibility of leukemic cells to the cytotoxicity of NKG2D-expressing cells." Leukemia **21**(10): 2103-2108.

Katsura, M., Furumoto, H., Nishimura, M., Kamada, M. and Aono, T. (1998). "Overexpression of CD44 variants 6 and 7 in human endometrial cancer." Gynecol Oncol **71**(2): 185-189.

Kostenko, S. and Moens, U. (2009). "Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology." Cell Mol Life Sci **66**(20): 3289-3307.

Kurosawa, S., Esmon, C. T. and Stearns-Kurosawa, D. J. (2000). "The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18." J Immunol **165**(8): 4697-4703.

Lanier, L. L. (2008). "Evolutionary struggles between NK cells and viruses." Nat Rev Immunol **8**(4): 259-268.

Larcher, C., Recheis, H., Sgonc, R., Gottinger, W., Huemer, H. P. and Irschick, E. U. (1997). "Influence of viral infection on expression of cell surface antigens in human retinal pigment epithelial cells." Graefes Arch Clin Exp Ophthalmol **235**(11): 709-716.

Laszik, Z., Mitro, A., Taylor, F. B., Jr., Ferrell, G. and Esmon, C. T. (1997). "Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway." Circulation **96**(10): 3633-3640.

Lawson, C. and Wolf, S. (2009). "ICAM-1 signaling in endothelial cells." Pharmacol Rep **61**(1): 22-32.

Leelayuwat, C., Townend, D. C., Degli-Esposti, M. A., Abraham, L. J. and Dawkins, R. L. (1994). "A new polymorphic and multicopy MHC gene family related to nonmammalian class I." Immunogenetics **40**(5): 339-351.

Lesley, J., Hyman, R. and Kincade, P. W. (1993). "CD44 and its interaction with extracellular matrix." Adv Immunol **54**: 271-335.

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D. and Darnell, J. (2000). "Molecular Cell Biology - 4th edition". W.H. Freeman, NY, New York. ISBN 0-7167-3136-3

Liu, Z., Guo, B. and Lopez, R. D. (2009). "Expression of intercellular adhesion molecule (ICAM)-1 or ICAM-2 is critical in determining sensitivity of pancreatic cancer cells to cytolysis by human gammadelta-T cells: implications in the design of gammadelta-T-cell-based immunotherapies for pancreatic cancer." J Gastroenterol Hepatol **24**(5): 900-911.

Lucas, S. and Coulie, P. G. (2008). "About human tumor antigens to be used in immunotherapy." Semin Immunol **20**(5): 301-307.

Machesky, N. J., Zhang, G., Raghavan, B., Zimmerman, P., Kelly, S. L., Merrill, A. H., Jr., Waldman, W. J., Van Brocklyn, J. R. and Trgovcich, J. (2008). "Human cytomegalovirus regulates bioactive sphingolipids." J Biol Chem **283**(38): 26148-26160.

Maeurer, M. J., Martin, D., Walter, W., Liu, K., Zitvogel, L., Halusczcak, K., Rabinowich, H., Duquesnoy, R., Storkus, W. and Lotze, M. T. (1996). "Human intestinal Vdelta1+ lymphocytes recognize tumor cells of epithelial origin." J Exp Med **183**(4): 1681-1696.

Mahvi, D. M., Carper, S. W., Storm, F. K., Teal, S. R. and Sondel, P. M. (1993). "Overexpression of 27-kDa heat-shock protein in MCF-7 breast cancer cells: effects on lymphocyte-mediated killing by natural killer and gamma delta T cells." Cancer Immunol Immunother **37**(3): 181-186.

Manjili, M. H., Wang, X. Y., MacDonald, I. J., Arnouk, H., Yang, G. Y., Pritchard, M. T. and Subjeck, J. R. (2004). "Cancer immunotherapy and heat-shock proteins: promises and challenges." Expert Opin Biol Ther **4**(3): 363-373.

Martino, A., Casetti, R., Sacchi, A. and Poccia, F. (2007). "Central memory Vgamma9Vdelta2 T lymphocytes primed and expanded by bacillus Calmette-Guerin-infected dendritic cells kill mycobacterial-infected monocytes." J Immunol **179**(5): 3057-3064.

McAdam, A. J., Schweitzer, A. N. and Sharpe, A. H. (1998). "The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells." Immunol Rev **165**: 231-247.

Meeh, P. F., King, M., O'Brien, R. L., Muga, S., Buckhalts, P., Neuberg, R. and Lamb, L. S., Jr. (2006). "Characterization of the gammadelta T cell response to acute leukemia." Cancer Immunol Immunother **55**(9): 1072-1080.

Mezger, M., Bonin, M., Kessler, T., Gebhardt, F., Einsele, H. and Loeffler, J. (2009). "Toll-like receptor 3 has no critical role during early immune response of human monocyte-derived dendritic cells after infection with the human cytomegalovirus strain TB40E." Viral Immunol **22**(6): 343-351.

Mizera-Nyczak, E., Dyszkiewicz, W., Heider, K. H. and Zeromski, J. (2001). "Isoform expression of CD44 adhesion molecules, Bcl-2, p53 and Ki-67 proteins in lung cancer." Tumour Biol **22**(1): 45-53.

Moody, D. B., Zajonc, D. M. and Wilson, I. A. (2005). "Anatomy of CD1-lipid antigen complexes." Nat Rev Immunol **5**(5): 387-399.

Moody, D. B. (2006). "TLR gateways to CD1 function." Nat Immunol **7**(8): 811-817.

Morini, M., Mottolese, M., Ferrari, N., Ghiorzo, F., Buglioni, S., Mortarini, R., Noonan, D. M., Natali, P. G. and Albini, A. (2000). "The alpha 3 beta 1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity." Int J Cancer **87**(3): 336-342.

Morino, M., Tsuzuki, T., Ishikawa, Y., Shirakami, T., Yoshimura, M., Kiyosuke, Y., Matsunaga, K., Yoshikumi, C. and Saijo, N. (1997). "Specific expression of HSP27 in human tumor cell lines in vitro." In Vivo **11**(2): 179-184.

Nachmani, D., Lankry, D., Wolf, D. G. and Mandelboim, O. (2010). "The human cytomegalovirus microRNA miR-UL112 acts synergistically with a cellular microRNA to escape immune elimination." Nat Immunol **11**(9): 806-813.

Odeberg, J., Browne, H., Metkar, S., Froelich, C. J., Branden, L., Cosman, D. and Soderberg-Naucler, C. (2003). "The human cytomegalovirus protein UL16 mediates increased resistance to natural killer cell cytotoxicity through resistance to cytolytic proteins." J Virol **77**(8): 4539-4545.

Oganesyan, V., Oganesyan, N., Terzyan, S., Qu, D., Dauter, Z., Esmon, N. L. and Esmon, C. T. (2002). "The crystal structure of the endothelial protein C receptor and a bound phospholipid." J Biol Chem **277**(28): 24851-24854.

Ohgitani, E., Kobayashi, K., Takeshita, K. and Imanishi, J. (1999). "Biphasic translocation of a 70 kDa heat shock protein in human cytomegalovirus-infected cells." J Gen Virol **80 (Pt 1)**: 63-68.

Orian-Rousseau, V. (2010). "CD44, a therapeutic target for metastasising tumours." Eur J Cancer **46**(7): 1271-1277.

Par, G., Rukavina, D., Podack, E. R., Horanyi, M., Szekeres-Bartho, J., Hegedus, G., Paal, M., Szereday, L., Mozsik, G. and Par, A. (2002). "Decrease in CD3-negative-CD8dim(+) and Vdelta2/Vgamma9 TcR+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection." J Hepatol **37**(4): 514-522.

Pascolo, S., Ginhoux, F., Laham, N., Walter, S., Schoor, O., Probst, J., Rohrlich, P., Obermayr, F., Fisch, P., Danos, O., Ehrlich, R., Lemonnier, F. A. and Rammensee, H. G. (2005). "The non-classical HLA class I molecule HFE does not influence the NK-like activity contained in fresh human PBMCs and does not interact with NK cells." Int Immunol **17**(2): 117-122.

Pende, D., Rivera, P., Marcenaro, S., Chang, C. C., Biassoni, R., Conte, R., Kubin, M., Cosman, D., Ferrone, S., Moretta, L. and Moretta, A. (2002). "Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity." Cancer Res **62**(21): 6178-6186.

Poccia, F., Battistini, L., Cipriani, B., Mancino, G., Martini, F., Gougeon, M. L. and Colizzi, V. (1999). "Phosphoantigen-reactive Vgamma9Vdelta2 T lymphocytes suppress in vitro human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines." J Infect Dis **180**(3): 858-861.

Poggi, A., Venturino, C., Catellani, S., Clavio, M., Miglino, M., Gobbi, M., Steinle, A., Ghia, P., Stella, S., Caligaris-Cappio, F. and Zocchi, M. R. (2004). "Vdelta1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by trans-retinoic acid." Cancer Res **64**(24): 9172-9179.

Qi, J., Zhang, J., Zhang, S., Cui, L. and He, W. (2003). "Immobilized MICA could expand human Vdelta1 gammadelta T cells in vitro that displayed major histocompatibility complex class I chain-related A-dependent cytotoxicity to human epithelial carcinomas." Scand J Immunol **58**(2): 211-220.

Qin, G., Mao, H., Zheng, J., Sia, S. F., Liu, Y., Chan, P. L., Lam, K. T., Peiris, J. S., Lau, Y. L. and Tu, W. (2009). "Phosphoantigen-expanded human gammadelta T cells display potent cytotoxicity against monocyte-derived macrophages infected with human and avian influenza viruses." J Infect Dis **200**(6): 858-865.

Raftery, M. J., Hitzler, M., Winau, F., Giese, T., Plachter, B., Kaufmann, S. H. and Schonrich, G. (2008). "Inhibition of CD1 antigen presentation by human cytomegalovirus." J Virol **82**(9): 4308-4319.

Raga, S., Julia, M. R., Crespi, C., Figuerola, J., Martinez, N., Mila, J. and Matamoros, N. (2003). "Gammadelta T lymphocytes from cystic fibrosis patients and healthy donors are high TNF-alpha and IFN-gamma-producers in response to Pseudomonas aeruginosa." Respir Res **4**: 9.

Rentala, S., Yalavarthy, P. D. and Mangamoori, L. N. (2010). "Alpha1 and beta1 integrins enhance the homing and differentiation of cultured prostate cancer stem cells." Asian J Androl **12**(4): 548-555.

Richard, J., Sindhu, S., Pham, T. N., Belzile, J. P. and Cohen, E. A. (2010). "HIV-1 Vpr up-regulates expression of ligands for the activating NKG2D receptor and promotes NK cell-mediated killing." Blood **115**(7): 1354-1363.

Robinson, J., Waller, M. J., Fail, S. C., McWilliam, H., Lopez, R., Parham, P. and Marsh, S. G. (2009). "The IMGT/HLA database." Nucleic Acids Res **37**(Database issue): D1013-1017.

Roland, C. L., Dineen, S. P., Toombs, J. E., Carbon, J. G., Smith, C. W., Brekken, R. A. and Barnett, C. C., Jr. (2010). "Tumor-derived intercellular adhesion molecule-1 mediates tumor-associated leukocyte infiltration in orthotopic pancreatic xenografts." Exp Biol Med (Maywood) **235**(2): 263-270.

Russano, A. M., Bassotti, G., Agea, E., Bistoni, O., Mazzocchi, A., Morelli, A., Porcelli, S. A. and Spinozzi, F. (2007). "CD1-restricted recognition of exogenous and self-lipid antigens by duodenal gammadelta+ T lymphocytes." J Immunol **178**(6): 3620-3626.

Rutella, S., Rumi, C., Puggioni, P., Barberi, T., Di Mario, A., Larocca, L. M. and Leone, G. (1999). "Expression of thrombospondin receptor (CD36) in B-cell chronic lymphocytic leukemia as an indicator of tumor cell dissemination." Haematologica **84**(5): 419-424.

Sanchez, V. and Dong, J. J. (2010). "Alteration of lipid metabolism in cells infected with human cytomegalovirus." Virology.

Sato, S., Miller, A. S., Howard, M. C. and Tedder, T. F. (1997). "Regulation of B lymphocyte development and activation by the CD19/CD21/CD81/Leu 13 complex requires the cytoplasmic domain of CD19." J Immunol **159**(7): 3278-3287.

Scheffer, G. L., Flens, M. J., Hageman, S., Izquierdo, M. A., Shoemaker, R. H. and Scheper, R. J. (2002). "Expression of the vascular endothelial cell protein C receptor in epithelial tumour cells." Eur J Cancer **38**(11): 1535-1542.

Shahgasempour, S., Woodroffe, S. B., Sullivan-Tailyour, G. and Garnett, H. M. (1997). "Alteration in the expression of endothelial cell integrin receptors alpha 5 beta 1 and alpha 2 beta 1 and alpha 6 beta 1 after in vitro infection with a clinical isolate of human cytomegalovirus." Arch Virol **142**(1): 125-138.

Sindhu, S. T., Ahmad, R., Morisset, R., Ahmad, A. and Menezes, J. (2003). "Peripheral blood cytotoxic gammadelta T lymphocytes from patients with human immunodeficiency virus type 1 infection and AIDS lyse uninfected CD4+ T cells, and their cytocidal potential correlates with viral load." J Virol **77**(3): 1848-1855.

Siu, D. (2009). "Cancer therapy using tumor-associated antigens to reduce side effects." Clin Exp Med **9**(3): 181-198.

Stephens, H. A. (2001). "MICA and MICB genes: can the enigma of their polymorphism be resolved?" Trends Immunol **22**(7): 378-385.

Stern-Ginossar, N., Elefant, N., Zimmermann, A., Wolf, D. G., Saleh, N., Biton, M., Horwitz, E., Prokocimer, Z., Prichard, M., Hahn, G., Goldman-Wohl, D., Greenfield, C., Yagel, S., Hengel, H., Altuvia, Y., Margalit, H. and Mandelboim, O. (2007). "Host immune system gene targeting by a viral miRNA." Science **317**(5836): 376-381.

Stevanovic, S. (2002). "Identification of tumour-associated T-cell epitopes for vaccine development." Nat Rev Cancer **2**(7): 514-520.

Sy, M. S., Guo, Y. J. and Stamenkovic, I. (1992). "Inhibition of tumor growth in vivo with a soluble CD44-immunoglobulin fusion protein." J Exp Med **176**(2): 623-627.

Ten Elshof, A. E., Brittenham, G. M., Chorney, K. A., Page, M. J., Gerhard, G., Cable, E. E. and Chorney, M. J. (1999). "Gamma delta intraepithelial lymphocytes drive tumor necrosis factor-alpha responsiveness to intestinal iron challenge: relevance to hemochromatosis." Immunol Rev **167**: 223-232.

Thomas, M. L., Samant, U. C., Deshpande, R. K. and Chiplunkar, S. V. (2000). "gammadelta T cells lyse autologous and allogenic oesophageal tumours: involvement of heat-shock proteins in the tumour cell lysis." Cancer Immunol Immunother **48**(11): 653-659.

Tsoyi, K., Jang, H. J., Nizamutdinova, I. T., Park, K., Kim, Y. M., Kim, H. J., Seo, H. G., Lee, J. H. and Chang, K. C. (2010). "PTEN differentially regulates expressions of ICAM-1 and VCAM-1 through PI3K/Akt/GSK-3beta/GATA-6 signaling pathways in TNF-alpha-activated human endothelial cells." Atherosclerosis **213**(1): 115-121.

Tsuneyoshi, N., Fukudome, K., Horiguchi, S., Ye, X., Matsuzaki, M., Toi, M., Suzuki, K. and Kimoto, M. (2001). "Expression and anticoagulant function of the endothelial cell protein C receptor (EPCR) in cancer cell lines." Thromb Haemost **85**(2): 356-361.

Tweedle, E. M., Khattak, I., Ang, C. W., Nedjadi, T., Jenkins, R., Park, B. K., Kalirai, H., Dodson, A., Azadeh, B., Terlizzo, M., Grabsch, H., Mueller, W., Myint, S., Clark, P., Wong, H., Greenhalf, W., Neoptolemos, J. P., Rooney, P. S. and Costello, E. (2010). "Low molecular weight heat shock protein HSP27 is a prognostic indicator in rectal cancer but not colon cancer." Gut **59**(11): 1501-1510.

Viey, E., Fromont, G., Escudier, B., Morel, Y., Da Rocha, S., Chouaib, S. and Caignard, A. (2005). "Phosphostim-activated gamma delta T cells kill autologous metastatic renal cell carcinoma." J Immunol **174**(3): 1338-1347.

Vomaske, J., Melnychuk, R. M., Smith, P. P., Powell, J., Hall, L., DeFilippis, V., Fruh, K., Smit, M., Schlaepfer, D. D., Nelson, J. A. and Streblow, D. N. (2009). "Differential ligand binding to a human cytomegalovirus chemokine receptor determines cell type-specific motility." PLoS Pathog **5**(2): e1000304.

Wainberg, Z., Oliveira, M., Lerner, S., Tao, Y. and Brenner, B. G. (1997). "Modulation of stress protein (hsp27 and hsp70) expression in CD4+ lymphocytic cells following acute infection with human immunodeficiency virus type-1." Virology **233**(2): 364-373.

Waldhauer, I. and Steinle, A. (2006). "Proteolytic release of soluble UL16-binding protein 2 from tumor cells." Cancer Res **66**(5): 2520-2526.

Wallace, M., Bartz, S. R., Chang, W. L., Mackenzie, D. A., Pauza, C. D. and Malkovsky, M. (1996). "Gamma delta T lymphocyte responses to HIV." Clin Exp Immunol **103**(2): 177-184.

Wang, X., Wang, E., Kavanagh, J. J. and Freedman, R. S. (2005). "Ovarian cancer, the coagulation pathway, and inflammation." J Transl Med **3**: 25.

Wang, X. Y., Kaneko, Y., Repasky, E. and Subjeck, J. R. (2000). "Heat shock proteins and cancer immunotherapy." Immunol Invest **29**(2): 131-137.

Wilkinson, G. W., Tomasec, P., Stanton, R. J., Armstrong, M., Prod'homme, V., Aicheler, R., McSharry, B. P., Rickards, C. R., Cochrane, D., Llewellyn-Lacey, S., Wang, E. C., Griffin, C. A. and Davison, A. J. (2008). "Modulation of natural killer cells by human cytomegalovirus." J Clin Virol **41**(3): 206-212.

Willcox, C. R., Pitard, V., Netzer, S., Salim, M., Couzi, L., Silberzhan, T., Moreau, J., Hayday, A. C., Willcox, B. E., Déchanet-Merville, J. (2010). "Identification of Endothelial Protein C Receptor (EPCR) as a TCR ligand for γδ T cells reactive against CMV-infected cells and tumor cells".
gamma delta T cell conference, Kiel, Germany

Wrobel, P., Shojaei, H., Schittek, B., Gieseler, F., Wollenberg, B., Kalthoff, H., Kabelitz, D. and Wesch, D. (2007). "Lysis of a broad range of epithelial tumour cells by human gamma delta T cells: involvement of NKG2D ligands and T-cell receptor- versus NKG2D-dependent recognition." Scand J Immunol **66**(2-3): 320-328.

Yanagisawa, N., Mikami, T., Mitomi, H., Saegusa, M., Koike, M. and Okayasu, I. (2001). "CD44 variant overexpression in gallbladder carcinoma associated with tumor dedifferentiation." Cancer **91**(2): 408-416.

Yang, Y., Lee, J. H., Kim, K. Y., Song, H. K., Kim, J. K., Yoon, S. R., Cho, D., Song, K. S., Lee, Y. H. and Choi, I. (2005). "The interferon-inducible 9-27 gene modulates the susceptibility to natural killer cells and the invasiveness of gastric cancer cells." Cancer Lett **221**(2): 191-200.

Yu, F., Ng, S. S., Chow, B. K., Sze, J., Lu, G., Poon, W. S., Kung, H. F. and Lin, M. C. (2010). "Knockdown of interferon-induced transmembrane protein 1 (IFITM1) inhibits proliferation, migration, and invasion of glioma cells." J Neurooncol.

Zheng, Z., Venkatapathy, S., Rao, G. and Harrington, C. A. (2002). "Expression profiling of B cell chronic lymphocytic leukemia suggests deficient CD1-mediated immunity, polarized cytokine response, altered adhesion and increased intracellular protein transport and processing of leukemic cells." Leukemia **16**(12): 2429-2437.

Zhu, H., Cong, J. P., Mamtora, G., Gingeras, T. and Shenk, T. (1998). "Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays." Proc Natl Acad Sci U S A **95**(24): 14470-14475.

Zocchi, M. R., Ferrarini, M., Migone, N. and Casorati, G. (1994). "T-cell receptor V delta gene usage by tumour reactive gamma delta T lymphocytes infiltrating human lung cancer." Immunology **81**(2): 234-239.

Zugel, U. and Kaufmann, S. H. (1999). "Role of heat shock proteins in protection from and pathogenesis of infectious diseases." Clin Microbiol Rev **12**(1): 19-39.

Zwirner, N. W., Dole, K. and Stastny, P. (1999). "Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes." Hum Immunol **60**(4): 323-330.