New Horizons in Translational Medicine 2 (2015) 159–166

Contents lists available at ScienceDirect

New Horizons in Translational Medicine

journal homepage: www.elsevier.com/locate/nhtm

Review article

Viral strategies to modulate NKG2D-ligand expression in Human Cytomegalovirus infection

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ARTICLE INFO

Available online 1 January 2016

Keywords:
Human cytomegalovirus (HCMV)
Natural Killer cells
Immune evasion
NKG2D
MICA
ULBP
UL16
UL142

ABSTRACT

Human cytomegalovirus is a paradigm for studies of viral strategies of immune evasion. In particular, the virus has developed multiple mechanisms for evasion of immune surveillance by lymphocytes expressing the activating receptor NKG2D. The human genome encodes several ligands able to bind NKG2D and in this article we review and discuss what is known about the various viral proteins and micro RNAs that act to minimise the recognition of the infected cell by modulation of the expression and trafficking of the different NKG2D ligand molecules.

Focal points:

- The activating receptor NKG2D is important in the immune response to HCMV.
- HCMV has developed multiple strategies to evade immunosurveillance by cytotoxic lymphocytes expressing NKG2D.
- A better understanding of immune evasion by HCMV will likely be relevant for the development of better vaccination strategies.

Contents

1. Introduction

Human Cytomegalovirus (HCMV) is a large, complex, double-stranded DNA virus that infects much of the world population. Although the immune system of a healthy individual can control the primary infection, it is not able to eliminate the virus and a persistent infection with occasional episodes of reactivation is established. Generally, in healthy people these infections are asymptomatic, however when the host is not fully immunocompetent, in early foetal life or individuals immunocompromised by immunodeficiency or by immunosuppression, HCMV infection/reactivation can lead to severe, life threatening disease [48]. Brief

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http://dx.doi.org/10.1016/j.nhtm.2015.11.002
2307-5023/

consideration of these observations leads to the conclusion that the ability of HCMV to persist in the infected host, in the face of a vigorous immune response, likely reflects the evasion of immune surveillance by the virus. Indeed, studies from many laboratories, over the last 4 decades have confirmed that much of the HCMV genome encodes gene products dedicated to subversion of nearly all aspects of the host immune response [20,48,84]. In this context, the number and variety of mechanisms employed by HCMV to prevent Natural Killer (NK) cell activation suggests, albeit indirectly, that these cytotoxic lymphocytes are particularly important in the immune response to HCMV [79].

NK cell recognition of target cells depends on a balance of signals coming from a range of inhibitory and activating receptors expressed at the NK cell surface [42] and multiple, different HCMV gene products modulate this recognition either by delivering substitute inhibitory signals (UL18 and UL40), or by modulating the expression of ligands for NK cell activating receptors directly (US2, US9, UL16, UL141, UL142, US18, US20, mIR -UL112-1) and indirectly (mIR US25-3p). It is striking that the majority of this array of HCMV strategies deployed to evade NK cell attack include amongst their targets the expression of ligands for the activating receptor NKG2D (Fig. 1).

NKG2D is a C-type lectin like receptor constitutively expressed, in humans, on all NK cells, CD8+αβ and γδ T cells. Generally, NKG2D is not found on the CD4+ T cells of healthy individuals, but its expression can be induced on subsets of CD4+T cells in disease [25], including HCMV infection [57]. The receptor is expressed at the cell surface as a homodimer and a charged amino-acid residue in the TM domain mediates interaction with a complementary-charged amino- acid in the signalling polypeptide DAP10 [82] that couples the NKG2D/NKG2D-ligand complex to the phosphatidylinositol-3- kinase (PI3K)/Grb-2/Vav1 pathway [65]. Engagement of NKG2D by its ligands triggers the activation of NK cell cytotoxicity, whereas for CD8+T cells, the interaction of the receptor with its ligands has a co-stimulatory function [7,31,61], unless the T cells have previously been activated, in which case ligation of NKG2D alone can trigger T cell activation [73].

While NKG2D is encoded by a single gene with only limited polymorphism, more than eight distinct ligands, some of them highly polymorphic, able to bind this receptor are encoded in the human genome. These ligands belong to two genetic families, both of which are encoded on chromosome 6: the highly polymorphic major histocompatibility complex class I-related chain (MIC) A and MICB genes that are found within the MHC region [5,7] and the UL16 binding proteins (ULBPs), also known as retinoic acid early transcripts (RAETs), that are located outside the MHC locus on the long arm of chromosome 6 [53]. The ULBP genes show relatively limited polymorphic variation [2]. Both the MICA/B and ULBP molecules show structural similarity to MHC class I proteins, however, they do not associate with β2-microglobulin nor do they present peptides [22]. The major structural differences between MICA/B and ULBP molecules are: 1) that the ULBPs lack an α3 domain, and 2) most MICA/B molecules are transmembrane proteins [25], with the exception of the MICA*008 allele, that like the ULBP-1, -2 and -3 molecules attaches to the plasma membrane via a Glycophosphatidylinositol (GPI)-anchor [4].

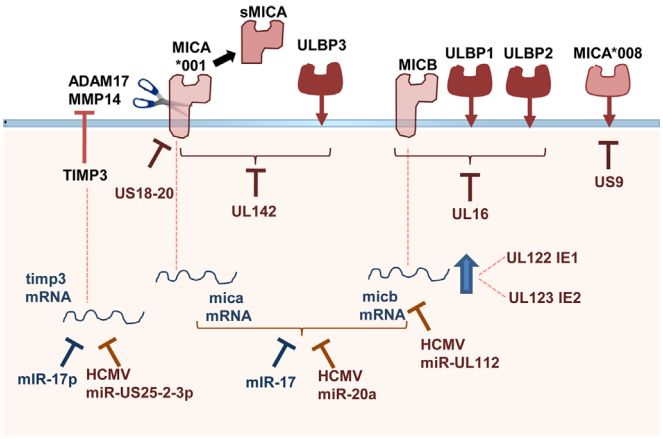

Fig. 1. Multiple HCMV encoded proteins and microRNAs are able to modulate NKG2D-ligand expression. Transcription of MICA and MICB mRNAs is affected by both cellular (blue) and HCMV-encoded (brown) microRNAs, as well as the gene products of the immediate early genes 1 and 2, UL122 and UL123. Surface expression of NKG2D-ligands is affected by the viral gene products UL16, UL142, US18-20, US9, and perhaps some other, still unidentified viral gene products. Release of soluble MICA (sMICA) by metalloproteases, such as MMP14 and ADAM17, is inhibited by cellular TIMP3 whose expression can, in turn, be regulated by cellular and viral microRNAs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Although mRNA encoding MICA/B and ULBP proteins has been found in some normal cells [15,58], it is generally accepted that, with the exception of actively proliferating cells [33], the expression of NKG2D-ligands on healthy cells and tissues is negligible. Instead, the expression of these molecules is upregulated in a range of pathologies including tumoral transformation, viral or bacterial infection and in autoimmunity [54]. Different stress stimuli lead to differential expression of the various NKG2D-ligands, depending on the cellular type and/or its metabolic status. For example, proteasome inhibition specifically upregulated ULBP2 in Jurkat cells [68], but ULBP1 in head and neck squamous cell carcinoma (HNSCC) cells [11]. Thus the regulation of the induction of expression is very complex and a full understanding of this process has not yet been achieved [54]. What is clear is that NKG2D-ligand expression is regulated at multiple levels including transcriptional, post-transcriptional, and post-translational (protein modifications, trafficking, and shedding). It is now also clear that HCMV deploys immune evasion mechanisms that target each step in the process of synthesis, traffic and surface expression of NKG2D-ligands by the infected cell (Fig. 1). Immune surveillance via the NKG2D receptor may be a particular problem for HCMV because the viral major immediate early (IE) proteins IE1 and IE2 potently activate transcription at the MICA/B and ULBP2 promoters [23,72] and increased expression of mRNA for a wide range of NKG2D-ligands can be detected in HCMV infected fibroblasts within 24hrs of infection [26,56,77]. However, despite this marked upregulation there are no changes in surface expression of NKG2D-ligands and HCMV-infected fibroblasts are actually protected from lysis [23,67], demonstrating that the virus can block NKG2D-ligand expression to evade NKG2D-dependent immune surveillance.

As mentioned above HCMV uses multiple mechanisms to avoid surface expression of NKG2D ligands by the infected cell (Fig. 1). The modulation of NKG2D-ligand mRNA species by HCMV encoded micro-RNAs has been comprehensively reviewed recently [24] so this aspect of immune evasion by HCMV will not be addressed here. Instead, this article will focus on a detailed overview of how HCMV targets NKG2D-ligand protein trafficking (UL16, UL142, US18/US20 and US9) and shedding (HCMV mIR US25-3p).

2. UL16

The UL16 glycoprotein of HCMV was the first viral immune evasion protein shown to target human ligands of the NKG2D receptor [15]. In fact, the ULBP (UL16-binding protein) family of ligands for the NKG2D receptor was discovered during a search for cellular ligands for this viral glycoprotein [15,64]. UL16 is a heavily glycosylated 50 kDa type I transmembrane protein, dispensable for growth *in vitro* and absent in the virions [35]. These observations suggested that this glycoprotein could have an immune regulatory