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Review

DNA Viruses and Genetic Modification of Dendritic Cells

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Abstract. An alternative approach to the use of tumor-peptide-loaded dendritic cells (DC) in immunotherapy would be the use of genetically modified DC using viral vectors expressing tumor-associated antigens (TAA). However, viruses have developed several immune escape mechanisms and, thus, one has to study the interaction between viruses and DC before these viruses can be used as an alternative strategy. Here we report that vaccinia virus (VV) as well as herpes simplex virus type 1 (HSV-1) are able to potently infect monocyte-derived DC, however, this infection leads to the inhibition of the DC-mediated T cell stimulation *in vitro*.

Key words: dendritic cells; viral vectors; gene therapy; vaccinia virus; herpes simplex virus.

Introduction

Dendritic cells (DC) are the most potent antigen--presenting cells, and in recent years several studies reported the use of modified DC in the field of immunotherapy. Encouraging results have been reported especially in melanoma studies. It also became clear that only mature DC induce potent immune responses, while immature DC induce tolerance. In the clinical studies reported so far, mainly peptide-loaded DC were used. An alternative approach would be the use of genetically modified DC using viral vectors encoding tumor--associated antigens (TAA) alone or combined with immuno-stimulatory molecules such as cytokines or costimulatory molecules. Nevertheless, since viruses have developed several immune evasion strategies, also targeting the function of DC, it is vital to understand the interaction between viruses and DC. In this review we will focus on DNA viruses.

Herpesviridae

The family of *Herpesviridae* consists of eight human herpes viruses: herpes simplex viruses (HSV-1 and HSV-2), cytomegalievirus (CMV), Epstein-Barr virus (EBV), varizella-zoster virus (VZV), and human herpes virus-6/7/8. These viruses are, as vaccinia virus (VV), double-stranded DNA viruses. So far only HSV have been developed as a versatile vector for gene therapy. Physiologically, the primary infections of HSV occur mucocutaneously in the mouth, face and eyes (HSV-1) or the genital region (HSV-2). After the acute infection, HSV establishes a persistent infection within sensory neurons. During this latency period, the viral genome persists within the neural nucleus in the absence of detectable viral protein expression[32]. The HSV genome comprises approx. 150 kb, encodes for about 70 viral proteins and is surrounded by an icosahedral nucleocapsid and a lipid layer envelope. The induction of the lytic pathway of HSV is triggered by the viral-en-

Abbreviations used: DC – dendritic cells, VV – vaccinia virus, HSV-1 – herpes simplex virus type 1.

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coded VP16 protein, which is carried into the infected cells as part of the tegumentum. This protein induces a cascade of gene expression, starting with immediate--early (IE) genes. These genes code for proteins, which induce viral DNA replication as well as intermediate and late viral gene expression.

As HSV can efficiently infect a number of different cell types, such as muscle, liver and especially neuronal cells, it has been developed as a versatile vector for gene therapy purposes[8]. Furthermore, HSV has the ability to package large genetic insertions, thus enabling the simultaneous expression of multiple TAA transcripts even in combination with immuno-modulators such as IL-2, IL-12 or CD80 and CD86. The virus binds to cells through interaction with heparin sulfate moieties followed by the binding of at least two viral glycoproteins, gB and gD, to specific surface receptors. Multiple HSV proteins are known to interfere with the generation of an anti-viral immunity. This might be desirable for gene therapy purposes where an anti-viral immunity reduces the longevity of the expression of the desired genes, but it severely limits the utility of wild--type HSV-1 vectors for immunotherapy strategies using transduced DC. HSV has developed several strategies to avoid humoral immunity, including the production of viral IgG Fc receptors at the surface of infected cells, which bind IgG and inhibit the Fc-dependent immune activation[31]. Additional escape strategies include the inhibition of human TAP by ICP47[30], which leads to a deficiency in antigen presentation, and interference with the chemokine system by secreting a broad spectrum of chemokine-binding protein[25].

For *in vivo* gene therapy purposes, the developed HSV vectors have specific properties, including the potency to kill target cells and the ability to form a tightly regulated latent infection. Still, there are major obstacles to overcome, which include safety problems, long-term gene expression, cytotoxicity, immunological competence of the host and the *in vivo* control of the vector[8]. In this respect the *ex vivo* transduction of DC represents an alternative approach and could possibly overcome some of the problems mentioned above. Nevertheless, it is vital to understand the interaction between DC and HSV in order to avoid an interference of the virus with the biological function of DC.

DC were generated from $CD14^+$ monocytes in the presence of GM-CSF and IL-4 under FCS free conditions (so called monocyte-derived DC). These immature DC were matured over a period of 4-5 days using a maturation cocktail containing IL-1ß, TNF-α, IL-6 and PGE_2. In our experiments, both immature as well as mature monocyte-derived DC cultured under serum--free conditions could be infected with HSV-1 wild--type virus[18]. DC express two of the necessary entry receptors Prr-1, an Ig superfamily member, and herpes virus entry mediator (Hve-A), a member of the TNF/nerve growth factor (NGF) family[26]. As a consequence of the receptor-mediated entry, a relatively low MOI of 1–5 is sufficient to transduce up to 90% of the cultured DC[18, 26]. Viral replication has only been demonstrated in immature DC[23] while mature DC do not support the generation of infectious viral particles[18]. Regarding the lytic properties of replicating HSV, this fact has a severe implication for the viability of the infected DC. While infected immature DC are prone to cell death, infected mature DC are stable for a relatively long period of time[18]. Generally, HSV has the capacity to hamper host cell protein synthesis by disruption of polysomes and degradation of mRNA[29]. This function is exhibited by the viral protein *vhs*, the product of the UL41 gene[19]. The upregulation of several DC surface molecules, such as MHC-II, CD86 and CD1a[26], after HSV infection are arguments against a complete host protein shut-off in DC. Similarly, the inhibition of monocytic cell function by HSV-1 infection seems to be independent of *vhs* expression[11]. Thus, the inability to induce maturation in HSV-infected immature DC[26] may be induced by a different, not yet identified viral gene product. In addition, apoptosis could also be partially responsible for this maturation block.

Furthermore, VP16 mediates the activation of JNK/SAPK and p38 in Vero cells[33] indicating an active interference with signal transduction pathways. However, whether these events contribute to the maturation blockade remains to be determined.

With regard to cytokine synthesis it has been shown that HSV-1-infected immature DC do not produce any cytokines with or without stimulation[26]. HAYWARD et al.[11] reported a reduced expression of costimulatory and adhesion molecules on the surface of infected monocytes, whereas MIKLOSKA et al.[23] reported a downregulation of CD1a, CD40, CD54, CD80 and CD86 on HSV-1-infected immature DC. Nevertheless, since the HSV-1 infection induces a high degree of cytopathicity in immature DC (20–45% of DC die within the first 24 to 48 h[23]), the observed downregulation could be due to cell death rather than a specific viral mechanism. Taken together, the work on the interaction of immature DC with HSV reveals an immuno-evasive strategy of the virus, since only mature DC can induce an anti-viral immunity.

Only fully mature DC induce potent immune responses[16], therefore, it might be crucial to transduce fully mature DC with genetically modified HSV vec-

tors. Mature DC are characterized by the expression of a specific array of surface molecules. These include costimulatory molecules such as CD40, CD80 and CD86, a high expression of MHC-I and MHC-II molecules and CD83. Unfortunately, the wild-type HSV-1 used so far impairs the allostimulatory capacity of mature DC[18], despite the fact that costimulatory molecules such as CD80 and CD86 as well as MHC-I and MHC-II molecules remain unaltered and are highly expressed on HSV-transduced DC.

However, CD83, the best known marker for mature DC, is completely degraded 10 h post infection, leading to a reduced capacity to stimulate allogeneic T cells. Interestingly, CD83-degradation occurs only in infected DC, uninfected bystander DC are not affected. Although the precise biological function of CD83 has not been defined so far, we could demonstrate that the inhibition of the nuclear export of CD83 mRNA from the nucleus into the cytoplasm leads to a reduction of the CD83 cell surface expression on DC, which is paralleled by a reduced allostimulatory capacity of these DC[17]. The CD83 degradation in HSV-1-infected DC is probably due to a viral-induced protein-degradation process[17].

For the future development of HSV-vectors for the *ex vivo* transduction of DC it is crucial to develop viral vectors with better safety profiles than the wild-type viruses and also to generate mutants, in which the gene responsible for the CD83 degradation is deleted.

Several second-generation vectors have already been developed but have not yet been used for DC transduction. These include HSV-deletion mutants displaying reduced neurovirulence[6] and increased safety[24] which have been designed and used for phase-I clinical trials to treat malignant glioma[21]. Furthermore, two vector systems with reduced lytic activity have also been developed a strain with deletion of the IE gene VP16, necessary for the initiation of the cascade of lytic gene expression[1], and, secondly, a vector system based on the cotransfection of a plasmid containing an HSV origin of replication and a packaging sequence with cosmids containing HSV genome but with a defective packaging sequence, termed HSV-1 amplicons[9, 27]. The latter system is currently under investigation in our laboratory.

Poxviridae

Three members of the *Poxviridae* family have so far been developed as vector systems for gene therapy purposes. One member of the *Orthopoxvirus* family, VV, which has been used as a poxvirus vaccination with remarkable efficacy and safety in the poxvirus eradication campaign, and two avipoxviruses (usually bird-restricted); the canarypoxvirus (ALVAC) and the fowlpoxvirus (FWPV). It has been demonstrated that FWPV is capable of infecting CD83-positive DC at a MOI of 10^4, and that a FWPV coding for recombinant CD80, CD54 and LFA-3 can be used to transduce DC thereby increasing their T cell activation[34]. Nevertheless, no further investigations regarding the phenotypic and functional properties of these infected DC have been performed so far.

ALVAC has been used in phase I clinical vaccination trials as a carrier for carcinoembryonic antigen without clinical side effects, suggesting a favorable safety profile[13]. However, for the transduction of DC, ALVAC seems not to be the perfect vector, since it induces apoptosis in immature and mature DC[14]. Therefore, additional vector systems have to be explored for direct DC transduction.

Here we will focus on VV as a representative of the *Poxviridae*. The VV system has been developed to a versatile vector system able to carry at least 25 kb of heterologous DNA[5]. In addition, several attenuated virus strains[28] and strains deficient for late gene expression have been developed[12]. Therefore, this also represents an interesting vector system to transduce DC. In these experiments the wild-type strain VV (wr) was used at a MOI of 2.5 to 5[15].

However, VV has developed even more immune evasion strategies than *Herpesviridae* (recently reviewed by ALCAMI and KOSZINOWSKI[2]). Still, VV has been further developed as an expression vector for gene therapy purposes[5, 20] as it is technically relatively easy to produce stable recombinant VV by homologous recombination.

Although the entry mechanism of VV into DC is not completely understood, a receptor-mediated process seems likely, as MOIs of 2.5 to 5 are sufficient for the transduction of both, immature and mature DC[7, 15]. After the transduction only genes regulated by early and, though to a far lesser degree, early-intermediate genes are expressed in monocyte-derived DC[15]. Due to the lack of late gene expression, no virus assembly and, consecutively, no cell lysis can be observed in infected DC. Nevertheless, the viability of infected DC is reduced due to the induction of apoptosis[15], presumably representing yet another mechanism of viral immune evasion. Interestingly, after the infection of immature DC with wild-type VV, DC cannot be matured anymore, neither by inflammatory maturation cytokines nor by other maturation stimuli such as LPS or Poly-I: C

(JENNE et al., unpublished observation). Thus, other known VV escape mechanisms, such as the syntheses of proteins that bind TNF or IL-1β[3], do not account for this maturation blockade.

Mature DC can be transduced with an efficacy equal to or slightly better than immature DC with both wild--type VV and MVA, a highly attenuated VV-strain[22], currently used in anti-cancer vaccinations, i.e. melanoma trials. Surprisingly, mature VV-infected DC are unable to stimulate the proliferation of allogeneic T cells[15], one of the key features of DC.

Comparable to HSV, VV induces the loss of CD83 cell surface expression on mature DC. This effect is initially seen only on infected DC, but after more than 48 h also partially on non-infected bystander cells[15].

Even more striking is the VV-induced effect regarding CD80, a costimulatory molecule of vital importance for the induction of potent immune responses[10]. It is very quickly downregulated on both infected DC and uninfected bystander DC[7, 15]. This bystander effect seems to be triggered by a viral protein, since the addition of brefeldin-A, an inhibitor of protein secretion, restores the CD80 expression on both infected and uninfected DC (JENNE et al., unpublished observation). Other costimulatory molecules, such as CD86 and CD40, remained unaltered. Furthermore, $CD4^+$ and $CD8^+$T cells cultured in the presence of wild-type VV-infected DC fail to proliferate.

Therefore, for the future development of VV-derived vectors it is vital to generate attenuated/mutated viral vectors which do not interfere with DC functions. In order to generate such vectors it is of vital importance to identify and characterize the viral gene products which interfere with DC functions by down-regulating relevant surface molecules, such as CD83 and CD80.

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