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Modified Vaccinia virus Ankara: Innate immune activation and induction of cellular signalling

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ABSTRACT

Attenuated poxviruses are currently under development as vaccine vectors against a number of diseases including, influenza, HIV, malaria and tuberculosis. Modified Vaccinia virus Ankara (MVA) is an attenuated, replication deficient vaccinia virus (VACV) strain which, similar to replication competent VACV, is highly immunogenic. The lack of productive viral replication further improves the safety profile of MVA as a vector, minimizing the potential for reversion to virulent forms particularly if used in immunocompromised individuals. Despite its inability to replicate in most mammalian cells, MVA still efficiently expresses viral and recombinant genes making it a potent antigen delivery platform. Moreover, due to the loss of various immunomodulatory factors MVA infection leads to rapid local immune responses, fulfilling a requirement of an adjuvant. In this review we take a look at the immunostimulatory properties of MVA, paying particular attention to the signalling of the innate immune system in response to MVA and VACV infection. Understanding the cellular and molecular mechanisms modulated by VACV will help in the future design and engineering of new vaccines and may provide insight into previously unknown mechanisms of dominant virus–host interactions.

1. Introduction

Modified Vaccinia virus Ankara (MVA) was developed as a highly attenuated strain of VACV during the last decades of the smallpox eradication campaign in the Institute for Infectious Diseases and Zoonoses (formerly the Institute of Medical Microbiology, Infectious and Epidemic Diseases) at the Ludwig-Maximilians-University of Munich. Chorioallantois vaccinia virus Ankara (CVA) was serially passaged in chicken embryo fibroblasts (CEF) in an attempt to restrict the broad host range of vaccinia virus in order to mimic more closely the host range restricted variola virus, the causative agent of human smallpox. After some 371 passages of CVA in CEF cells Mayr and Munz [1] reported the acquisition of distinct growth characteristics in eggs, tissue culture and in laboratory animals. Subsequent passages of this strain yielded a virus with a highly attenuated phenotype which was designated MVA [2]. Due to its improved safety profile, MVA was chosen by the Bavarian State Vaccine Institute in Munich and licensed for safer immunization against smallpox. This licensed MVA vaccine was used in over 100,000 individuals without any occurrence of the severe adverse reactions that were associated with the application of conventional VACV vaccines [3,4].

The molecular basis of the attenuation of MVA was first glimpsed after analysis of the virus genome revealed that 15% of the original CVA genome was lost in six large deleted regions [5]. Further analysis of the complete sequence of the MVA genome demonstrated that many of the genes that are affected by deletions and mutations are important regulators of VACV-host interaction [6]. As a result MVA displays a greatly restricted host range and is no-longer capable of replicating in human and many other mammalian cells, however despite this many viral and recombinant genes are efficiently expressed in infected cells, making MVA a potent viral vector [7]. One of the first recombinant MVA vaccines carried the hemagglutinin and nucleoprotein of influenza virus. In a murine model, immunization with this recombinant MVA induced antigen-specific cellular and humoral immunity that protected against lethal influenza virus challenge [8]. The success of this recombinant MVA vaccine prompted the development of numerous others, for in depth reviews see [9–14].

2. Induction of interferon

The potent immunostimulatory properties of MVA were first observed by Mayr et al. [2] who showed that intraperitoneal administration of MVA to mice enhanced the clearance of carbon particles from the blood after 2 days, indicating an increase in phagocytotic

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activity of immune cells. Additionally, intranasal inoculation of rabbits induced "serum interferons" which inhibited Sindbis virus replication in an *in vitro* infection assay.

Type I and II interferons are critical mediators of the host antiviral response, their importance in VACV infection is demonstrated by the synthesis and secretion of soluble IFN-binding proteins by VACV infected cells [15–17]. This ability to antagonize host interferon signalling is not possessed by MVA which unlike other VACV strains induces type I IFN in mice and in bone marrow-derived plasmacytoic dendritic cells [18].

3. Induction of chemokines and cell migration

The engagement of pattern recognition receptors (PRR) by viral components and the production of pro-inflammatory cytokines and chemokines is an important initial step in the induction of antiviral immunity. Chemokines coordinate the recruitment of leukocytes and thus play a crucial role in bridging the innate and adaptive immune responses.

Infection of human monocytic cells induces a robust chemokine response, with a marked upregulation of CCL2, CCL3, CCL4, CXCL8 and CXCL10. Utilizing an *in vitro* chemotaxis assay, we observed that infection with MVA resulted in the production of soluble chemotactic factors for monocytes, T cells and NK cells. Further antibody inhibition studies demonstrated that CCL2 induces chemotaxis in the human monocytic cell line THP-1. The importance of CCL2 *in vivo* was then investigated in a murine intranasal infection model, where we showed that inoculation with MVA but not other VACV strains triggers immigration of monocytes, neutrophils and CD4+ lymphocytes into the lung, which is accompanied with strong expression of CCL2. Crucially inoculation of CCL2 deficient mice showed that CCL2 plays an important role in the early immigration of leukocytes to the site of infection [19].

An important aspect of antiviral immunity is the development of the CD8+ T cell response, in which chemokines play an important role. In an interesting study by Duffy *et al.* the authors reported a novel source of virus specific CD8+ T cells that are primed in the bone marrow after intradermal MVA infection. Priming of these cells required the presence of phagocytic myeloid cells, however the antigen was delivered to the bone marrow inside recirculating neutrophils. The migration of these neutrophils from the dermis to the bone marrow was dependant on CCR1 [20].

Another study by Kastenmüller et al. provides some insight into the development of the CD8+ memory T cell response after MVA infection in the lymph node. They observed that CD8+ memory T cells are predominantly concentrated in the interfollicular area and close to the high endothelial venules, a positioning that allows the rapid encounter of pathogen infected cells spread *via* the lymphatics. Upon re-infection, the recruitment of antiviral memory CD8+ T cells is further optimized by local production of CXCL9 and CXCL10 [21].

4. Induction of cellular signalling

Currently the prevailing theory is that a molecular structure of the VACV virion is recognized by TLR2 leading to the induction of the host immune response. The first indication of the involvement of TLR2 came from Zhu et al. [22]. Using bone marrow derived dendritic cells (BMDC) they proposed that the production of pro-inflammatory cytokines, IL1 and IL-6, upon VACV infection was dependent on the TLR2/MyD88 pathway, whereas the production of IFN-β was MyD88 independent. Infection of TLR2$^{-/-}$, MyD88$^{-/-}$ and IFNαβR$^{-/-}$ mice confirmed these results *in vivo* and they then went on to demonstrate the importance of both pathways in the differentiation of DC and the activation of T cell responses [22]. This first indication of the involvement of TLR2 prompted many others to examine the role of TLR2 in VACV immunity. However, the results of many of these studies are somewhat contradictory, and as yet there is no clear consistent message as to the role played by TLR2 recognition in VACV immunity.

In an important study by Barbalat et al. [23] it was reported that inflammatory monocytes (IM) produce IFN-β in response to viral but not bacterial TLR2 ligands. Using a model to selectively deplete IM they demonstrated the importance of IM for the production of IFN-β *in vivo*, however, a notable omission from this paper is that they did not use the TLR2$^{-/-}$ mice to demonstrate the requirement of TLR2 for IFN-β production *in vivo*. Thus, as the definitive evidence, which would contradict the findings of Zhu et al. [22], that TLR2 is not important for the *in vivo* production of IFN-β, is not presented it is difficult to reconcile these two studies. Additionally in the study by Barbalat et al. the use of inhibitors to block endocytosis also blocked the production of IFN-β in response to VACV, which was interpreted as demonstrating the requirement of internalization of TLR2 after ligation. However UV inactivated viruses, as used in this study, still enter the cell by endocytosis, which is also blocked by these inhibitors. Thus a similar experiment by Delaloye et al. in THP-1 cells was interpreted as demonstrating the requirement of virus internalization for production of IFN-β, which was subsequently shown to be due to recognition by the MDA-5/IPS-1 pathway [24].

An important factor with many of these studies is the use of UV inactivated virus to demonstrate TLR2 dependence. VACV encodes a myriad of host immunomodulatory proteins, which interfere with multiple immune pathways including TLR signalling pathways [25]. Thus the use of an inactivated virus does not accurately simulate the situation *in vivo* due to the lack of these viral factors, which have profound effects on the host immune response. Furthermore residual viral gene expression may actually amplify the host immune response to UV treated virus in a way that is not normally seen with live virus. This is a phenomenon that we have routinely observed particularly with replication competent VACV. For instance, infection of THP-1 cells with VACV Wyeth treated with 0.25–2 J of UV increases CCL2 expression, which is only prevented by UV dosages at 4 J (Fig. 1). This increase of chemokine expression is presumably due to the loss of virus-host interaction factors, which would normally block this activation. It remains to be determined whether a virus treated in this manner is capable of evoking long lasting immune protection.

To date one of the best indications as to a possible role for TLR2 comes from O'Gorman et al. [26] who found that VACV induces rapid activation of the STAT3 signalling pathway in DC and T-cells through production of IL-6 in a TLR2 dependent manner. However to their surprise they found that despite this rapid activation of STAT3, TLR2 did not affect the outcome of mouse pox infection and there were no differences in the viral burden during the early stages of infection with VACV. It was only after 6 days that TLR2$^{-/-}$ and IL-6$^{-/-}$ mice were found to have an increased viral burden and lower levels of anti-VACV antibodies. Despite these differences, the viral loads converged by day 15 indicating that the infections resolved in a similar timeframe. A possible explanation for this is that whilst CD8 T-cell responses to VACV are dependent on MyD88, the upstream receptor on CD8 T-cells is not TLR2, and TLR2$^{-/-}$ mice produce a robust CD8 T-cell response similar to that of wild type mice after infection [27].

When it comes to host immune response stimulation, it is apparent that there are pronounced differences between VACV strains. MVA is distinct from other VACV strains in its ability to activate NF-κB, and activation of NF-κB by MVA is dependent on double-stranded RNA activated protein kinase (PKR) [28]. Similarly it was shown that early MVA gene expression rapidly induces phosphorylation of ERK2, an event that precedes NF-κB activation