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A single vaccination with non-replicating MVA at birth induces both immediate and long-term protective immune responses

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ABSTRACT

Newborns are considered difficult to protect against infections shortly after birth, due to their ineffective immune system that shows quantitative and qualitative differences compared to adults. However, here we show that a single vaccination of mice at birth with a replication-deficient live vaccine Modified Vaccinia Ankara [MVA] efficiently induces antigen-specific B- and T-cells that fully protect against a lethal Ectromelia virus challenge. Protection was induced within 2 weeks and using genetically modified mice we show that this protection was mainly T-cell dependent. Persisting immunological T-cell memory and neutralizing antibodies were obtained with the single vaccination. Thus, MVA administered as early as at birth induced immediate and long-term protection against an otherwise fatal disease and appears attractive as a new generation smallpox vaccine that is effective also in children. Moreover, it may have the potential to serve as platform for childhood vaccines as indicated by measles specific T- and B-cell responses induced in newborn mice vaccinated with recombinant MVA expressing measles antigens.

1. Introduction

The inefficiency of the immune system in newborns has been a major bottleneck to develop safe and effective vaccines at this age. Only BCG (in HIV negatives) and Hepatitis B vaccine are recommended at birth, while others are given later during infancy (e.g. rotavirus, inactivated poliovirus vaccine during the first 12 months, measles/mumps/rubella vaccine at 12 months or older). Importantly, multiple doses during infancy/childhood are required in all cases to induce high levels of protection [1]. Consequently, vaccination of neonates (within the first 4 weeks) and/or a reduced and more effective schedule in infants would be a major advance. This is true not only for the reduction of mortality and morbidity associated with childhood diseases, but also for all infectious diseases that occur in young children with an often faster and more severe form than in adults, such as AIDS, malaria, tuberculosis and also smallpox.

In fact, smallpox was one of the biggest killers in human history prior to its eradication, with an estimated death toll of more than 300 million in the 20th century alone [2]. Children and especially infants younger than 12 months were the main victims with death rates up to 80 percent compared to 30 percent in adults. Due to the immunologic cross-reactivity between orthopoxviruses, it was possible to eradicate smallpox using replicating vaccinia virus. Storage of smallpox causing variola virus is confined to only two highly contained repositories, but modern molecular technology and recent findings of active variola virus in freezers outside those containments raise concerns about accidental or intentional release of the virus. Moreover, since the vaccination programs ceased more than 40 years ago, the majority of people has never been vaccinated, resulting in a high level of susceptibility. Zoonotic orthopoxvirus infections with vaccinia, cowpox, camelpox and monkeypox virus are increasing [3]. Similar to smallpox, most fatalities of monkeypox virus infections occur in children [4].

Why are the young more vulnerable? It is generally accepted that newborns mount mainly Th2 biased T-cell responses and produce no or only low levels of antibodies with limited affinity; in addition, these responses are of shorter duration than in adults [5–9]. However, under certain circumstances such as activation of pattern recognition receptors or during certain viral infections, newborn mice can mount protective T-cell responses over time, indicating the potential for neonatal immunization [10]. Different experimental vaccines [11–18] were shown to induce efficient immune responses when administered in one week old mice or

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even at birth. However, only attenuated replicating vaccines induced protection against lethal infections that were generally effective after several immunizations and thus at a stage with a progressed immunological maturity. Hence, replicative vaccines require substantial time to induce successful protection, and the risk of uncontrolled disseminated infections by the vaccines still represents major limitations [19–22].

Modified Vaccinia Ankara virus (MVA) fails to replicate in mammals including humans and is safe even in immune compromised hosts [23]. It is highly immunogenic in humans [24] and its efficacy has been proven in several smallpox animal models such as Ectromelia virus (ECTV), rabbitpox or monkeypox [25–27]. Another major advantage of MVA is its capacity to support the genetic insertion of several antigens [28] that potentially induce protection against other infectious diseases. ECTV is believed to be a superior mouse infection model, firstly because it mimics variola infections in humans with a fatal outcome upon low dose exposure via the respiratory route [29]. Secondly because as a natural mouse pathogen, it causes infection following a natural course where the readout is not restricted to e.g. viral load in lung at a particular day, or the use of immunocompromised mice, which limits the characterization of vaccine responses in several infectious disease indications. Data generated in the ECTV model have successfully supported licensure of MVA-BN® in Europe and Canada, underscoring the acceptance of this model.

We have previously demonstrated that the vaccination of mice with MVA at birth is safe and induces an increase of FLT3 ligand, leading to an accelerated development of plasmacytoid dendritic cells (pDC) and an improved resistance against heterologous viral infection in the first week of life [30]. Here, we show that a single vaccination of mice with MVA at birth not only induces innate, but also adaptive immune responses including effector and long-term memory T-cells as well as neutralizing antibody responses. Importantly, within two weeks after vaccination the adaptive immune response fully protected mice against a lethal intranasal challenge with ECTV.

Moreover, using measles as an example for a common childhood disease, we show that vaccination of newborn mice with recombinant MVA expressing measles antigens induced measles specific T and B-cell responses that were also at comparable levels as in adult mice, indicating a potential for the use of recombinant MVA as childhood vaccine platform.

2. Materials and methods

2.1. Mice

Time-mated C57BL/6J and BALB/c female mice were purchased from Harlan Winkelmann. B-cell receptor/T11μMT transgenic mice, AID$^{-/-}$, MHC class I/β2m$^{-/-}$, T-cell receptor βδ$^{-/-}$ and FLT3$^{-/-}$ mice on a C57BL/6 background were obtained from the University of Zurich or were bred at Bavarian Nordic. Litters were of mixed sex. All animal experiments were performed in compliance with the German Animal Welfare Law. All studies were approved by the government of Upper Bavaria.

2.2. Vaccines and challenge virus

The MVA used was MVA-BN® (European Collection of Cell Cultures [ECACC] V00083008). Recombinant MVA-Measles vaccine (Fig. 5 only) uses the same MVA-BN® vector and encodes the Fusion-, Hemagglutinin- and Nucleo-proteins of measles virus. ECTV strain Moscow was obtained from ATCC (VR-1374). All viruses were produced by Bavarian Nordic, and propagated and titrated as described previously [26].

2.3. Immunization and challenge

Mice were immunized at the indicated doses of MVA subcutaneously with 50 μl of viral suspension within 6–24 h after birth or at the age of 8 weeks. Animals were bled and sacrificed at different time points and spleens were processed for flow cytometric analyses. For ECTV challenge, mice were anaesthetized with ketamine/xylamine and virus was applied intranasally at the indicated doses in a volume of 25 μl, apart from 2-week old animals that received 12.5 μl. 7-week old mice were challenged with 3×10^4 $TCID_{50}$ ECTV [31], 2- and 4-week old mice at the doses indicated. Post-challenge, body weight was monitored daily, responsiveness at least twice daily for 21 days. Unresponsive mice were sacrificed. To evaluate immunogenicity of the childhood vaccine candidate MVA-Measles, MVA-Measles was administered twice three weeks apart.

2.4. ECTV plaque assay

ECTV plaque assay was used to determine the viral load in lungs, which were homogenized and titrated on Vero C1008 cells using fourfold serial dilutions starting at 1:100. After 3 days incubation and a crystal violet staining (Sigma Aldrich), the titer was calculated from the first dilution step that revealed a mean plaque number ≤150.

2.5. Vaccinia antibody responses

Vaccinia-specific serum IgG titers were measured by ELISA and neutralizing titers by plaque reduction neutralization test (PRNT) as described previously [25].

2.6. Flow cytometry and ELISpot

After erythrolysis, a part of the splenocytes was incubated 5 h with B8R-peptide (5 μg/ml TSYKFESV, Coring) or without as control, in the presence of BD GolgiPlug™. Cells were stained with anti-CD8-eFluor™-450, anti-CD4-eFluor™-780, anti-CD44-FITC, anti-CD62L-PercP-Cy5.5, anti-CD127-APC, anti-IFNγ-PE-Cy7 (all eBioscience) and anti-Granzyme B-PE (Invitrogen). Intracellular staining was performed using Cytofix/Cytoperm™ (BD Biosciences). Flow cytometric data were obtained using an LSR II and analyzed with FlowJo (Tree Star). The remaining splenocytes were stimulated 20 h with or without B8R or YAMGVGVELEN peptides (5 μg/ml, Coring) and IFNγ-secreting cells were detected by ELISpot (BD Biosciences).

2.7. Statistical analysis

Statistical analysis was performed using Excel 2010, Sigmaplot 12 and StatXact 11, with common statistical tests as stated in the figure legends.

3. Results

3.1. Neutralizing antibodies as well as effector and long-term memory T-cells are induced by MVA in newborn mice

Newborn mice were immunized at birth with the standard dose of MVA (1×10^8 $TCID_{50}$) identified as optimal for adult animals [26,27] and people [24], or a lower dose (2×10^6 $TCID_{50}$) previously used in newborn mice [30]. At regular intervals, vaccinia-specific IgG was determined by ELISA (Fig. 1a). In adult mice, antibodies were detectable seven days following a single immunization with 1×10^8 $TCID_{50}$ of MVA and reached a plateau

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