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Live-attenuated bivalent measles virus-derived vaccines targeting Middle East respiratory syndrome coronavirus induce robust and multifunctional T cell responses against both viruses in an appropriate mouse model

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ABSTRACT

Cases of Middle East respiratory syndrome coronavirus (MERS-CoV) continue to occur, making it one of the WHO´s targets for accelerated vaccine development. One vaccine candidate is based on live-attenuated measles virus (MV) vaccine encoding the MERS-CoV spike glycoprotein (MERS-S). MV_{vac2}-MERS-S(H) induces robust humoral and cellular immunity against MERS-S mediating protection. Here, the induction and nature of immunity after vaccination with MV_{vac2}-MERS-S(H) or novel MV_{vac2}-MERS-N were further characterized. We focused on the necessity for vector replication and the nature of induced T cells, since functional $CD8^+$ T cells contribute importantly to clearance of MERS-CoV. While no immunity against MERS-CoV or MV was detected in MV-susceptible mice after immunization with UV-inactivated virus, replication-competent MV_{vac2}-MERS-S(H) triggered robust neutralizing antibody titers also in adult mice. Furthermore, a significant fraction of MERS CoV-specific $CD8^+$ T cells and MV-specific $CD4^+$ T cells simultaneously expressing IFN-γ and TNF-α were induced, revealing that MV_{vac2}-MERS-S(H) induces multifunctional cellular immunity.

1. Introduction

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a member of the *Coronaviridae* family and emerged in 2012 in the Kingdom of Saudi Arabia (Zaki et al., 2012). Coronaviruses typically cause mild infections of the upper respiratory tract, but already in 2002, the severe acute respiratory syndrome CoV (SARS-CoV) with a mortality rate of about 10% among infected patients was introduced in the human population. SARS-CoV spread world-wide and caused more than 8000 diagnosed infections, but was contained within a year after its emergence (http://www.who.int/csr/sars/country/table2004_04_21/en/).

In contrast, infections with MERS-CoV are ongoing for more than 5 years, with 2103 laboratory-confirmed cases distributed over 27 countries with at least 733 deaths that were reported to the WHO by November 2017 (http://www.who.int/emergencies/mers-cov/en/). This apparent case-fatality rate of 35% is of grave concern, because epidemic spread as has been observed for SARS-CoV could result in a disastrous death toll. MERS-CoV has been introduced zoonotically by transmission from dromedary camels to human patients (Alagaili et al., 2014; Haagmans et al., 2014; Reusken et al., 2013a) and serological studies indicate wide-spread and early distribution among this animal host (Alagaili et al., 2014; Reusken et al., 2013b). Therefore, a continuous risk of transmission especially to persons in close contact to camels is evident. Fortunately, the human to human transmission rate has remained low. Aside of individuals with regular contact to camels, only health care workers or relatives of MERS-CoV patients have a considerable risk of infection (Alraddadi et al., 2016; Drosten et al., 2014), but still at a modest level. Nonetheless, the high case fatality rate, the recurrent outbreaks of MERS-CoV infections, and especially the risk of virus adaptation potentially resulting in epidemic or even pandemic spread make the development of an effective vaccine against MERS-CoV an international priority.

The efficacy of several vaccine candidates has been demonstrated in different animal models up to even dromedary camels (reviewed in (Okba et al., 2017)). One of these candidates, MV_{vac2}-MERS-S(H) (Malczyk et al., 2015), is based on the measles virus (MV) vaccine platform technology (Mühlebach, 2017), and encodes the MERS-CoV spike protein (S) as an additional antigen in the backbone of recombinant MV_{vac2} (del Valle et al., 2007) resembling vaccine strain

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Moraten that is authorized and in use in the US since 1968. This candidate induces both robust humoral and functional cellular immune-responses against MERS-CoV. Moreover, MERS-CoV viral load and inflammation of the lung were significantly reduced in challenged mice that had been vaccinated with MV_{vac2}-MERS-S(H), before (Malczyk et al., 2015).

While these experiments provided proof of concept for efficacy of this vaccine candidate, further mechanistic insights into the nature of the induced T cell responses remain to be elucidated. These are of special interest, since it has been shown that T cells are essential for clearance of the infection (Coleman et al., 2017; Zhao et al., 2014): Depletion of $CD8^+$ T cells increased overall inflammation, bronchiolar inflammation, lymphocyte infiltration, and pleuritis at day 7 post-infection in mice (Coleman et al., 2017), while MERS CoV-susceptible mice depleted of all T cells were unable to clear the virus (Zhao et al., 2014). As an alternative to the spike glycoprotein, conserved (internal) structural proteins such as the nucleocapsid protein N are of special interest as putative target of anti-viral T cell responses to be triggered by future MERS vaccines (Agnihothram et al., 2014).

Therefore, we have also generated and characterized MERS-CoV N protein-encoding vaccine candidates based on the MV_{vac2} vaccine platform, in this study. To further characterize the induction of MERS CoV-specific immune responses, we first analyzed the necessity for viral replication for the induction of MERS CoV- and MV-specific immune responses using the highly immunogenic MV_{vac2}-MERS-S(H) vaccine candidate. In addition, we characterized the functionality of $CD8^+$ and $CD4^+$ T cell responses in juvenile (6–12 week old) and adult (7 months of age) mice using flow cytometry and functional assays.

2. Material and methods

2.1. Cells

Vero (African green monkey kidney) (ATCC# CCL-81) and 293 T (ATCC CRL-3216) cell lines were purchased from ATCC (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM, Biowest, Nuaillé, France) supplemented with 10% fetal bovine serum (FBS; Biochrom, Berlin, Germany) and 2 mM L-glutamine (L-Gln; Biochrom). JAWSII dendritic cells (ATCC CRL-11904) were purchased from ATCC and cultured in MEM-α with ribonucleosides and deoxyribonucleosides (GIBCO BRL, Eggenstein, Germany) supplemented with 20% FBS, 2 mM L-Gln, 1 mM sodium pyruvate (Biochrom), and 5 ng/mL murine GM-CSF (Peprotech, Hamburg, Germany). DC2.4 murine dendritic cells (Shen et al., 1997) were cultured in RPMI containing 10% FBS, 2 mM L-Gln, 1% non-essential aminoacids (Biochrom), 10 mM HEPES (pH 7,4), and 50 µM 2-Mercaptoethanol (Sigma-Aldrich, Steinheim, Germany). Cells were cultured at 37 °C in a humidified atmosphere containing 6% CO_2 for a maximum of 6 months of culture after thawing of the original stock.

2.2. Plasmids

The codon-optimized gene encoding MERS-CoV-N (Genebank accession no. JX869059) flanked with *Aat*II/*Mlu*I binding sites in plasmid pMA-RQ-MERS-N was obtained by gene synthesis (Invitrogen Life Technology, Regensburg, Germany). The antigen and the immediate early cytomegalovirus (CMV) promoter (Martin et al., 2006) were inserted into plasmids p(+)BR-MV_{vac2}-ATU(P) (del Valle et al., 2007) or p(+)MV_{vac2}-GFP(H) via *Mlu*I/*Aat*II and *Sfi*I/*Sac*II, respectively, to generate p(+)PolII-MV_{vac2}-MERS-N(P) or p(+)PolII-MV_{vac2}-MERS-N(H). For construction of lentiviral transfervectors encoding MERS-CoV-N, the ORF of MERS-N was amplified by PCR with primers encompassing flanking restriction sites *Nhe*I/*Xho*I and template pMA-RQ-MERS-N. Details on primers and PCR are available upon request. PCR products were cloned into pCR2.1-TOPO (Invitrogen Life Technology) and fully sequenced. Intact antigen ORF was cloned into pCSCW2gluc-IRES-GFP (Hewett et al., 2007) using *Nhe*I/*Xho*I restriction sites to yield pCSCW2-MERS-N-IRES-GFP.

2.3. Production of lentiviral vectors and generation of antigen-expressing dendritic cell lines

Lentiviral vectors were produced and used for the generation of antigen-expressing dendritic cell lines as described, before (Malczyk et al., 2015). In short, HIV-1-derived vectors were generated using a standard 3 plasmid system and the transfer vector plasmid pCSCW2-MERS-N-IRES-GFP by PEI transfection. Subsequent purification after harvest of transfected 293 T cells yielded virus stocks used to transduce DC cell lines, which were single cell-sorted by FACS and selected for antigen expression.

2.4. Viruses

MERS-N encoding vaccine candidates MV_{vac2}-MERS-N(P) and MV_{vac2}-MERS-N(H) were rescued as described (Malczyk et al., 2015; Martin et al., 2006). Single syncytia were picked and overlaid onto 50% confluent Vero cells cultured in 6-well plates and harvested as "passage 0" (P0) by scraping and freeze-thaw cycle of cells at the time of maximal infection. Subsequent passages were generated as described for the following viruses. MERS-N encoding vaccine viruses in P3 were used for characterization, viruses in P4 for vaccination. MERS-S encoding vaccine virus MV_{vac2}-MERS-S(H), and control virus MV_{vac2}-ATU(P) (Malczyk et al., 2015) were also used in P4 for vaccination. Both as well as MV_{vac2}-GFP(P) and MERS-CoV (isolate EMC/2012) (Zaki et al., 2012) used for neutralization assays were propagated and titrated on Vero cells by the method of Spearman and Kaerber (Hubert, 1984; Kärber, 1931). MV_{vac2}-MERS-S(H) was inactivated by UV-irradiation using a CL-1000 UV crosslinker (UVP, Cambridge, UK). 100 µL of virus suspension in 48-well-plates on ice were exposed to UV light of 254 nm at 3 cm distance from the UV source of $1{,}85 \times 10^5$ µJ/cm^2 for 30 min. Inactivation of virus was controlled by incubation of Vero cells with a control aliquot inactivated, in parallel. All virus stocks were stored in aliquots at −80 °C.

2.5. Western blot analysis

Cells were lysed and immunoblotted as previously described (Funke et al., 2008). A rabbit anti-MERS-CoV serum (1:1000) was used as primary antibody for MERS-CoV-N and a rabbit anti-MV-N polyclonal antibody (1:25,000) (Abcam) for MV-N detection. A donkey HRP-coupled anti-rabbit IgG (H&L) polyclonal antibody (1:10,000) (Rockland, Gilbertsville, PA) served as secondary antibody for both. Peroxidase activity was visualized with an enhanced chemiluminescence detection kit (Thermo Scientific, Bremen, Germany) on Amersham Hyperfilm ECL (GE Healthcare, Freiburg, Germany).

2.6. Animal experiments

All animal experiments were carried out in compliance with the regulations of German animal protection laws and as authorized by the RP Darmstadt. Six- to 12-week-old or 7 months old $IFNAR^{-/-}$-CD46Ge mice (Mrkic et al., 1998) deficient for type I IFN receptor and transgenically expressing human CD46 were inoculated intraperitoneally (i.p.) with 1×10^5 $TCID_{50}$ of recombinant viruses or UV-inactivated vaccine preparations on days 0 and either on day 21 or 28. Mice were bled on days 0, 28, and 49 post initial infection (p.i.). Serum samples were stored at − 20 °C. Mice were euthanized on days 32, 42, or 49 p.i., and splenocytes were harvested for assessment of cellular immune responses.

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