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Development of an animal model of progressive vaccinia in nu/nu mice and the use of bioluminescence imaging for assessment of the efficacy of monoclonal antibodies against vaccinial B5 and L1 proteins

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ABSTRACT

Bioluminescence imaging (BLI) was used to follow dissemination of recombinant vaccinia virus (VACV) expressing luciferase (IHD-J-Luc) in BALB/c nu/nu mice treated post-challenge with monoclonal antibodies (MAbs) against L1 and B5 VACV proteins in a model of Progressive Vaccinia (PV). Areas Under the flux Curve (AUC) were calculated for viral loads in multiple organs in individual mice. Following scarification with 10^5 pfu, IHD-J-Luc VACV undergoes fast replication at the injection site and disseminates rapidly to the inguinal lymph nodes followed by spleen, liver, and axillary lymph nodes within 2–3 days and before primary lesions are visible at the site of scarification. Extension of survival in nude mice treated with a combination of anti-B5 and anti-L1 MAbs 24 h post challenge correlated with a significant reduction in viral load at the site of scarification and delayed systemic dissemination. Nude mice reconstituted with 10^4 T cells prior to challenge with IHD-J-Luc, and treated with MAbs post-challenge, survived infection, cleared the virus from all organs and scarification site, and developed anti-VACV IgG and VACV-specific polyfunctional $CD8^+$ T cells that co-expressed the degranulation marker CD107a, and IFNγ and TNFα cytokines. All T cell reconstituted mice survived intranasal re-challenge with IHD-J-Luc (10^4 pfu) two months after the primary infection. Thus, using BLI to monitor VACV replication in a PV model, we showed that anti-VACV MAbs administered post challenge extended survival of nude mice and protected T cell reconstituted nude mice from lethality by reducing replication at the site of scarification and systemic dissemination of VACV.

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1. Introduction

Concerns that the agent of smallpox (variola virus) might be used as a bioterrorist weapon have prompted the US government to embark on a program of vaccine acquisition and to prepare for large scale vaccination in the event of a smallpox outbreak. In the US, the licensed cell culture-based smallpox vaccine, ACAM2000, replaced the previously used calf lymph-based Dryvax® vaccine for the immunization of military personnel, designated first responders, and those involved in orthopoxvirus research (Wharton et al., 2003). Studies with ACAM2000 vaccine indicated that it closely matches the safety profile of Dryvax in both non-clinical and clinical trials (Greenberg and Kennedy, 2008). Following skin scarification, the vaccine virus establishes local limited infection that leads to a robust cellular and humoral responses resulting in a long term protective immunity. However, in individuals with underlying immune deficiencies, excessive viral replication can lead to serious adverse events such as generalized vaccinia, eczema vaccinatum, and progressive vaccinia (PV), and could be life threatening disease (Beachkofsky et al., 2010; Bray and Wright, 2003; Frey et al., 2009; Lane and Goldstein, 2003). Screening for predisposing conditions

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cannot completely prevent the exposure of at-risk individuals to smallpox vaccine either as a result of inadvertent vaccination or through household contact (Redfield et al., 1987; Vora et al., 2008). The management of PV cases in humans is challenging. Since 1955, only vaccinia immune globulins (VIG) therapy has been approved for the treatment of severe side effects associated with smallpox vaccination (Bray and Wright, 2003; Cono et al., 2003; Wittek, 2006). A recent case report described a member of the U.S. military with underlying cancer who was inadvertently vaccinated with ACAM2000 vaccine resulting in a life threatening complication of PV. High doses of vaccinia immune globulin intravenous (VIGIV; 6–24,000 U/kg) together with investigational antiviral drugs ST-246 and brincidofovir were used to achieve viral clearance and ultimately the patient was cured (Lederman et al., 2012). The accident highlighted the need to identify potent antivirals and develop sensitive animal models for testing of treatments for PV and other smallpox vaccine-related complications.

In the course of infection, vaccinia virus produces at least two types of virions that differ in their function and antigenic profile; intracellular mature virion (MV) and extracellular enveloped virion (EV) (Smith et al., 2002). Animal studies and analysis of plasma from smallpox vaccine recipients identified several important antigen targets in MV and EV that are recognized by immune sera and have led to the development of polyclonal sera and monoclonal antibodies (MAb) for post exposure treatments as potential replacements for VIGIV. Antibodies against the MV proteins A27, H3, and L1, and against the EV proteins A33 and B5, were shown to inhibit the spread of vaccinia virus and variola virus *in vitro* and protected mice infected with VACV from lethality when administered before or after challenge (Chen et al., 2006, 2007; Galmiche et al., 1999; Ramirez et al., 2002). In animal models, combinations of antibodies targeting both MV and EV were shown to be more effective than individual antibodies (Lustig et al., 2005; McCausland et al., 2010). Two new monoclonal antibodies, M12B9 and h101 MAb, that recognize MV L1 protein and EV B5 protein respectively, were recently developed (Benhnia et al., 2009; Kaever et al., 2014). The murine anti-L1 MAb M12B9 showed significant protection of normal mice against respiratory challenge with the Western Reserve strain of VACV (WR VACV) (Kaever et al., 2014). The human anti-B5 MAb h101 protected normal mice from intranasal challenge with WR VACV and immune deficient mice from intravenous challenge with ACAM2000 (Benhnia et al., 2009; Crickard et al., 2012). A MAb against MV H3 protein combined with anti-B5 MAb h101 have shown extension of survival in immune deficient SCID mice infected intravenously with $VACV_{NYCBOH}$ vaccine strain (McCausland et al., 2010).

Currently, PV disease is modeled in athymic nu/nu (nude) mice (no T cells) or in SCID mice with severe immune deficiency (no T cells and no B cells). In these models, mice are subjected to intracutaneous infection via scarification at the base of the tail or to tail scarification with VACV or with smallpox vaccines. Animals are observed for development of skin lesions, weight loss, and lethality. Viral loads are measured *in vitro* in skin explants and in isolated organs. In nu/nu mice infected with the Lister strain of VACV, viral DNA could be detected at the scarification site skin and in internal organs on day 2 and day 30 post-infection, respectively, using PCR analysis (Neyts et al., 2004). In SCID mice scarified with Dryvax vaccine, lesions were noted starting from day 4 post infection (Fisher et al., 2011). In both studies, the effect of antiviral treatments on disease progression was followed by measuring the size of the primary lesion and by enumerating lesions at distal sites (Fisher et al., 2011; Neyts et al., 2004). Although these experiments identified promising therapies for the treatment of PV, they employed methods that provided only limited assessment of virus dissemination and of the effectiveness of antivirals especially at early time points after infection. Such information is crucial for the selection of optimal treatments for the prevention of PV.

Previously, we described the use of bioluminescence imaging

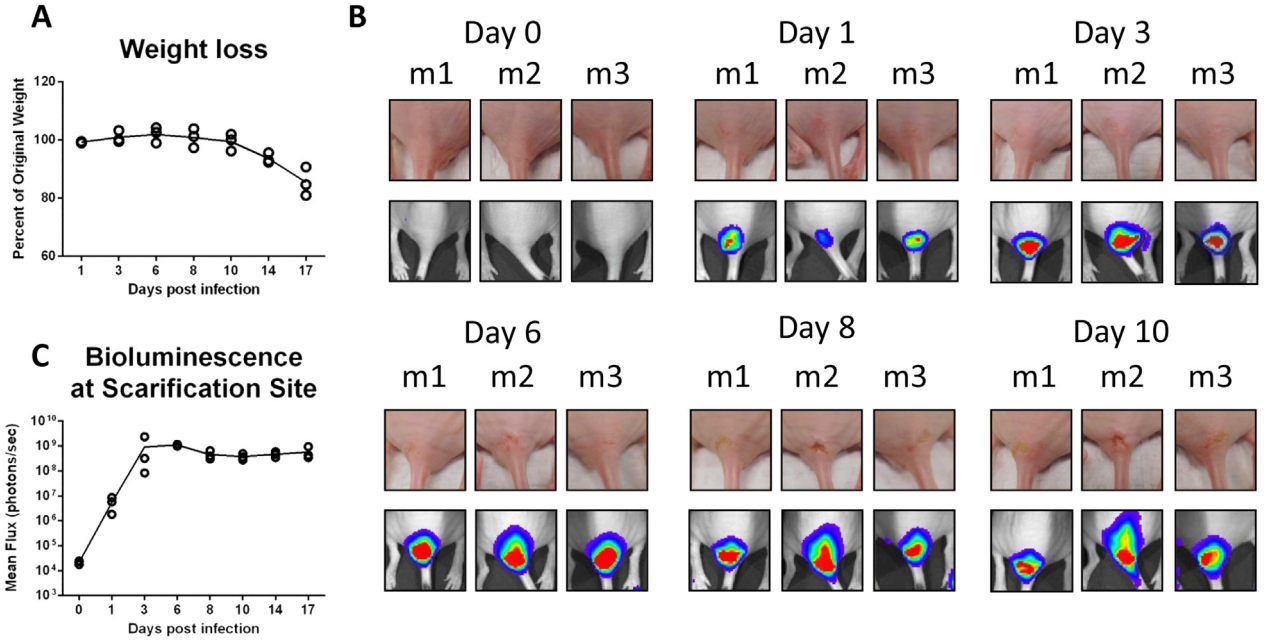

Fig. 1. Detection and quantification of IHD-J-Luc by bioluminescence imaging. BALB/c nu/nu mice were infected with IHD-J-Luc VACV at 10^5 pfu/mouse via scarification and were observed for weight loss (A), and for development of primary lesion and for presence of bioluminescence signal at the site of scarification between Day 0 and Day 17 post-infection (B). Total fluxes at the scarification site were determined in individual mice and used to calculate mean total flux ± S.D. (C). Panels in B show sequential images of scarification sites of infected mice m1, m2, and m3 recorded with a digital camera (upper panels) and by IVIS 50 instrument (lower panels) on Day 0 (prior to infection) and on Days 1, 3, 6, 8, and 10 post-infection. Solid lines in panels A and C show mean values averaged from three mice used in the experiment; each circle represents one animal. The experiment was performed twice with similar results.