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Induction, treatment and prevention of eczema vaccinatum in atopic dermatitis mouse models

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ABSTRACT

Eczema vaccinatum is a severe and occasionally lethal complication of smallpox vaccine, characterized by systemic viral dissemination, distant from the initial inoculation site of the vaccine. A major risk factor for eczema vaccinatum is a background of atopic dermatitis, a chronic, common allergic, relapsing disorder, manifested by dry and inflamed skin, itchy rash, Th2 biased immune response and hypersensitivity to various antigens. Unlike the severe manifestations of eczema vaccinatum in humans, current models present only mild symptoms that limits examination of potential therapeutics for eczema vaccinatum.

The atopic dermatitis and eczema vaccinatum models we present here, are the first to simulate the severity of the diseases in humans. Indeed, dermatitic mice display persistent severe dermatitis, characterized by dry and inflamed skin with barrier dysfunction, epidermal hyperplasia and significant elevation of serum IgE. By exposing atopic dermatitis mice to ectromelia virus, we generated eczema vaccinatum that mimic the human disease better than known eczema vaccinatum models. Similarly to humans, eczematous mice displayed enlarged and disseminated skin lesions, which correlated with elevated viral load. Cidofovir and antiviral antibodies conferred protection even when treatment started at a late eczematous stage. Moreover, we are the first to demonstrate that despite a severe background of atopic dermatitis, modified vaccinia Ankara virus (MVA) vaccination protects against lethal ectromelia virus exposure. We finally show that protection by MVA vaccination is dependent on $CD4^+$ T cells and is associated with significant activation of $CD8^+$ cytotoxic T cells and induction of humoral immunity.

1. Introduction

Atopic dermatitis (AD) is a chronic relapsing skin disease of complex etiology characterized by erythematous and pruritic rash affecting approximately 20% of children in developed countries. Active AD persists in most cases up to age 5, yet certain percentage of AD patients suffer from life-long relapsing AD symptoms [1,2]. Scaling of the skin, hypersensitivity to foreign antigens and aberrant activity of the immune system, are only few of several abnormalities leading to Th2 biased immune response, accompanied by systemic allergic manifestations in AD patients. The aberrant immune response and the defects in dermal integrity present a major risk for AD patients to develop disseminated infections such as eczema herpeticum or eczema vaccinatum (EV) following exposure to herpes or vaccinia viruses (VACV) [2].

Vaccination with live VACV was used to eradicate smallpox. Today, immunization is recommended only for laboratory workers and first responders. AD is a contra-indication for vaccination due to the risk of EV. Yet, occasional EV cases appear involving long period of hospitalization and treatment with repeated high doses of VIG and antivirals [2–4].

EV is the most common severe pathology associated with smallpox vaccination that occurs in roughly 10–40 cases per million of either VACV immunized humans, or following a contact with vaccinee [5–7]. A background of active or history of AD have been associated with most cases of EV. Severe cases of EV involve virus

Abbreviations: EV, eczema vaccinatum; AD, atopic dermatitis; DNFB, 1-Fluoro-2,4-dinitrobenzene; ECTV, ectromelia virus; CDV, cidofovir; p.i., post infection; VACV, vaccinia virus; TEWL, trans epidermal water loss; MVA, modified vaccinia Ankara virus; VIG, vaccinia virus immunoglobulin; α-VACV, rabbit anti-VACV hyper immune serum; s.c., subcutaneously; i.n., intranasal; i.m., intramuscular; H&E, hematoxylin–eosin; BRP, biological reference preparation; MTTD, mean time to death; PRNT, plaque reduction neutralization test; d, day; h.p.i, hour post infection.

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dissemination and occasionally multi-organ failure with mortality rates of 30% when untreated [2].

Current animal models of EV, based on mild AD, exhibit only local skin lesions with rarely disseminated rash and does not involve systemic spread, unlike the human disease [7,8]. These limitations highlight the need to develop additional models of severe EV with disseminated phenotype in order to evaluate treatment.

In this study we established a robust AD model in mice by modifying a protocol of repeated sensitization with the hapten 1-Fluoro-2,4-dinitrobenzene (DNFB) on the back skin of two mouse strains, Nc/Nga and SKH-1 [9–11]. Nc/Nga mice are known for their vulnerability to develop AD either spontaneously or following allergen treatment [12,13]. SKH-1 mice are widely used in dermatologic research as immunocompetent and hairless mice, which allow easy manipulation of the skin as well as easy visualization of the cutaneous response [14].

Here, we show that DNFB-sensitized mice develop persistent severe dermatitis, characterized by very dry and red skin, loss of epithelial integrity manifested by an increase in the trans epidermal water loss (TEWL), epidermal hyperplasia and significant elevation in serum IgE levels. We further induced EV in AD mice by infection with ectromelia virus (ECTV) that resulted in severe disseminated rash, high viral load in the skin and internal organs with mortality rates of about 60–100%. These robust EV models were used to assess the therapeutic efficacy of Modified Vaccinia Ankara virus (MVA) vaccination and the therapeutic value of VACV-immunoglobulin (VIG), cidofovir (CDV) and anti-VACV hyper immune serum (α-VACV). We show that the best therapeutic treatment is a combination of CDV and α-VACV, which abrogated the viral load in skin and in internal organs, completely diminished skin lesions and prevented death. Delayed treatment (starting 8 days post infection, d.p.i.) of already eczematous mice, required high dose CDV treatment. Finally we show that despite the allergic AD background, pre-or post-exposure immunization with the smallpox vaccine MVA, is safe and protects against lethal respiratory poxvirus infection.

2. Materials and methods

2.1. Mice

Six-week-old female NC/Nga (Japan SLC, Shizuoka) or SKH-1 (Charles River, Germany) mice were housed in filter-top individual caging systems (IVC). General procedures for animal care and housing were done in compliance with the regulations for animal experiments at the Israel Institute for Biological Research. The end-points were weight loss (25% of the initial weight) and/or inability to respond to the righting reflex. Animals that reached these predetermined end-points were humanely sacrificed by cervical dislocation or by CO_2. ECTV challenge and MVA vaccination were performed on anesthetized mice (Ketamine 75 mg/kg, Xylazine 7.5 mg/kg in PBS).

2.2. Cells and viruses

Vero (ATCC CCL-8) and BS-C-1 (ATCC CCL-26) cells were maintained as recommended by ATCC.

Ectromelia strain Moscow (ATCC VR-1374) was propagated and tittered as described previously [15]. MVA clonal isolate F6 [16] at the 584th CEF passage was used and titrated as described previously [17].

2.3. Allergen sensitization and virus challenge

AD-like skin lesions were induced by repeated application of 100 μl of 0.15–0.45% DNFB (Sigma-Aldrich, Israel) in acetone/olive oil (3:1) to the back skin of mice once a week for 9 weeks prior to ECTV challenge and once a week for two weeks after the challenge. Control groups were treated with vehicle only (vehicle-sensitized).

ECTV infection: anesthetized mice were administrated subcutaneously (s.c.) with 100pfu (100 μl) or intranasally (i.n.) with 5000pfu (20 μl).

MVA vaccination regimen: DNFB-sensitized mice (7th treatment), were vaccinated intramuscularly (i.m.) with 1×10^8 (50 μl) PFU prime and boosted 3 weeks later. Two weeks after boost the mice were challenged with ECTV. Additional groups were vaccinated once at the indicated day. Vehicle-sensitized mice received the same regimen of MVA vaccination.

2.4. Measurements of TEWL and serum IgE

After the 9th DNFB-sensitization, total serum IgE level was quantified by ELISA (BD Pharmingen) according to manufacturer protocol. Trans epidermal water loss (TEWL) was measured using an evaporimeter (Tewameter TM300, Courage-Khazaka, Cologne, Germany). Water loss through the skin was measured with an open chamber probe applied to the skin. Baseline TEWL was measured in vehicle-treated mice.

2.5. Histopathologic analysis

Dorsal skin specimens were collected following the 9th DNFB treatment, 7 or 12 days after ECTV challenge, fixed in 4% formalin and embedded in paraffin. Skin sections (5 μm) were cut and stained with either hematoxylin–eosin (H&E) or stained for virus using rabbit anti VACV polyclonal serum followed by anti-rabbit Alexa fluor 555-conjugated Ab, according to manufacturer instructions (Thermo Fisher Scientific). Tissue sections were evaluated at magnifications of X100 or X400.

2.6. EV scoring

Severity of lesion-progression was scored between 0 to 4 as follows: No lesions (0). Local lesion, smaller than 0.5 cm in diameter (1). Local lesion larger than 0.5 cm with or without few distant lesions (2). Disseminated lesions (3) or (4) depending on rash severity.

2.7. Determination of viral load in mouse organs and blood

Seven days post viral challenge, mice were bled. Liver, spleen, lung and skin were collected and kept at −80 °C until analyzed. Organs were weighted, homogenized and viral load was determined by titration on BS-C-1 cells as previously described [18].

2.8. VIG, BRP and rabbit anti vaccinia hyper immune serum (α-VACV)

VIG (Omr IgG-am 5% VIG 50 mg IgG/ml, Omrix biopharmaceuticals Ltd.). BRP, a pharmacopeia European reference standard (human VIG BRP (biological reference preparation)) batch 1 was used to standardize the antibody titers to IU/ml. BRP potency is 23 IU/ml.

Hyper immune rabbit anti WR serum (α-VACV) was prepared as previously described [19].

2.9. Plaque reduction neutralization test (PRNT), comet inhibition and ELISA

PRNT was previously described [20]. Comet inhibition assay was performed as described previously [21]. ELISA to determine anti-vaccinia titer in the VIG, serum and BRP was done as described previously. Briefly, ELISA plates were adsorbed with βPL-inactivated

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