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Protective murine and human monoclonal antibodies against eczema vaccinatum

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Abstract

Background—Eczema vaccinatum is the most common severe pathology associated with smallpox vaccination (vaccinia virus), occurring at high rates among individuals with a previous history of atopic dermatitis (atopic eczema).

Methods—Monoclonal antibodies capable of neutralizing vaccinia virus, anti-H3 and anti-B5, have been developed as a potential therapy for treatment of human eczema vaccinatum.

Results—Using a small animal model of eczema vaccinatum, here we demonstrate that both murine and fully human monoclonal antibodies effectively limit eczema vaccinatum disease, foreshortening both the disease kinetics and the severity of the erosive viral skin lesions.

Conclusions—These neutralizing antibodies would likely be effective at reducing or eliminating clinical disease in people with eczema vaccinatum or other severe side effects of the smallpox vaccine.

Introduction

Over the past decade there has been intensive interest in understanding immunity to poxviruses at multiple levels, with much of the effort focused on understanding the potent immunity elicited by the smallpox vaccine and the requirements for protection against smallpox and related pathogenic poxviruses [1]. From a clinical perspective, updating and stockpiling smallpox vaccine has been a major focus [2], as has been the development of anti-smallpox therapeutics. Two categories of anti-smallpox therapeutics have been extensively explored for potential biodefense use against smallpox itself or rare severe side effects of smallpox vaccination: small molecule drugs [3,4] [5] and monoclonal antibodies [6–9].

A clonal isolate of VACV_{NYCBOH}, ACAM2000, has now been developed as a cell culture derived smallpox vaccine, with a comparable immunogenicity and safety profile to Dryvax [2]. Smallpox vaccine “take” for classic VACV_{NYCBOH}, ACAM2000, or Lister is observed

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as the formation of a pustule starting on approximately day 5 post-vaccination and lasting for 1–2 weeks thereafter [10,11]. The vaccine provides outstanding immunity, but causes a variety of side effects that have been reason for concern [12]. Common side effects include fever and satellite pocks (additional pustules near the primary pustule) [11,12]. More severe side effects include progressive vaccinia, generalized vaccinia, encephalitis, vaccinia keratitis, and eczema vaccinatum [12]. Eczema vaccinatum is a severe and difficult to treat pathology that occurs in some immunized humans with a previous history of atopic dermatitis (eczema). Currently, VIG (Vaccinia Immune Globulin) is the only licensed therapeutic to treat severe side effects of smallpox vaccination [13]. In addition, VIG has shown efficacy against smallpox itself, in clinical trials in the early 1960s [14]. Unfortunately, VIG is a poorly characterized, variable human product that is of limited potency [13,14]. These limitations of VIG have led to great interest in the development of an alternative high potency anti-vaccinia and anti-smallpox immunotherapy.

Poxviruses have two distinct virion forms, Intracellular Mature Virions (MV, IMV) and Extracellular Enveloped Virions (EV, EEV), each with different biology. The two virion forms do not share any surface proteins, and therefore the virion forms are immunologically distinct and are not neutralized by antibody of a single specificity [1]. VIG contains both anti-MV and anti-EV antibodies [15–17]. Therefore, we have pursued development of a VIG replacement therapeutic product containing one anti-MV mAb and one anti-EV mAb, each capable of neutralizing their respective virion form [6]. We first identified murine and human neutralizing antibodies against the MV surface antigen H3 [16,17], and EV surface antigen B5 [7,18], both of which neutralize in the presence of complement. We further demonstrated that B5 antibodies are highly effective at catalyzing complement mediated killing of VACV infected cells as an additional antiviral mechanism [7,18]. Recently, we demonstrated that anti-H3 and anti-B5 murine and human mAbs were significantly more effective than VIG at extending lifespan of immunodeficient SCID mice infected with VACV [6]. As a second model of treatment efficacy, we have now examined the efficacy of anti-H3 and anti-B5 mAbs against VACV in a new mouse model of eczema vaccinatum.

Materials and Methods

Viruses

VACV_{WR} (Western Reserve) stocks were grown as described [16,17]. The ACAM2000 stock was generated by single passage amplification in HeLa cells of ACAM2000® vaccine virus (Acambis, UK).

Mouse infections

NC/Nga mice were used in all experiments [19]. Eczematous skin lesions were induced as described previously [20]. Briefly, back skin were shaved and dermatitis was induced by 2 rounds of treatment with *Dermatophagoides farinae* extract (Der f, Greer Laboratories) and Staphylococcal enterotoxin B (SEB) (Sigma-Aldrich). During this treatment, the back skin was occluded with a bandage that was removed the following week. Clinical scores of eczematous skin lesions are based on severity (0, no signs; 1, mild; 2, intermediate; 3, severe) of four signs (redness, bleeding, eruption and scaling). Scoring of skin lesions was performed in a blind manner. VACV (Western Reserve strain or ACAM2000, 10⁶ PFU per 3 µl) was dropped on the center of skin lesions of mice with a clinical score of ≥7 (eczematous group) followed by pricking 15 times with a 27G needle. In some experiments, a cohort (normal group) of age and sex-matched mice with healthy skin was also infected at the same anatomical site. After VACV infection on day 0, mice were monitored daily in a blind manner and body weight and size of the skin lesion were measured. Murine anti-VACV mAbs anti-B5 #B126 and/or anti-H3 #41 were intraperitoneally or intravenously

injected 24 hours before or 12 hours after VACV infection. Animals were housed in micro-isolator cages in ventilated racks. All animal experiments were conducted using the Institutional Animal Care and Use Committee approved animal protocols.

Antibodies

Anti-B5 IgG2a clone B126 and anti-H3 IgG2a clone 41 were generated and produced as described [6,18]. Fully human monoclonal antibodies h101 and hV26 were generated and produced as described [6,7].

Statistical analysis

Statistical analysis was carried out using Prism 5.0 software (Graphpad). Size of skin lesions, incidence and changes of body weight were analyzed with unpaired, two-tailed T-test. Cumulative skin lesion burden (e.g. Fig. 2B) was calculated as the area under the curve (AUC) of skin lesion size over time and analyzed by unpaired, two-tailed T-test. $P < 0.05$ was considered statistically significant. Disease incidence (prevalence) statistical significance was determined by Fisher's Exact Test.

Results

NC/Nga eczema vaccinatum model

Eczema vaccinatum is a severe, potentially fatal, side effect of smallpox vaccination that occurs in some vaccinees with a previous history of eczema. The reason for this striking and peculiar pathology of VACV has been unclear. Previously, no small animal model of this clinical disease had been available. We recently developed a mouse model of eczema vaccinatum [21]. NC/Nga mice are susceptible to atopic dermatitis [19,20]. In this system, NC/Nga mice are shaved and atopic dermatitis is induced with allergen (Figure 1A–B). Eczematous mice are then infected intradermally with vaccinia virus (VACV_{WR}), and virus-induced skin lesions are observed (Figure 1C). Viral titers in the skin lesions are > 100-fold increased in eczematous mice [21], and the disease is strongly associated with defective NK cell activity and elevated IL-17 levels [21]. The infection and eczema vaccinatum lesions are self resolved after two weeks (Figure 2A). Eczema vaccinatum can also be induced with vaccine strain viruses, though the skin lesions are less severe, consistent with the low replicative potential of VACV_{NYCBOH} related strains (e.g., ACAM2000) in mice (Figure 1D).

Protective efficacy of single murine anti-VACV mAbs

The protective efficacy of murine mAbs against VACV at treating eczema vaccinatum was first tested in a pre-exposure model, wherein eczematous mice were injected with a single 200 µg anti-H3 or anti-B5 mAb one day prior to intradermal infection with VACV. It was unclear whether antibodies would be effective against intradermal infection with VACV, particularly given the defective NK cells, which are important for early control of the virus. Development of skin lesions was delayed in mAb treated mice (Figure 2A, C) and severity of the lesions was substantially reduced (anti-B5, $P < 0.01$ at all timepoints day 2 to 14. Anti-H3, $P \leq 0.01$ at all timepoints day 2 to 13, except day 3. $P < 0.05$ at days 3 and 14.) (Figure 2A). Disease incidence was moderately reduced, from 100% to 80% in anti-H3 or anti-B5 treated mice (Figure 2C). Lesion disease burden was significantly reduced after treatment with either anti-H3 mAb ($P < 0.001$) or anti-B5 mAb ($P < 0.001$). Minimal change in body weight was observed in all groups (Figure 2D), consistent with our previous work [21].

Post-exposure prophylaxis efficacy of the antibodies was then tested. Eczematous mice were infected intradermally with VACV. Intravenous injection of anti-H3 or anti-B5 mAb was

done 12 hours after infection. Anti-B5 treatment was still able to delay lesion development ($P < 0.05$, day 3)(Figure 3A, C), reduce the disease incidence (Figure 3C), and reduce the overall lesion disease burden ($P < 0.05$)(Figure 3B). Anti-H3 was not able to reduce disease incidence when provided after infection (day 3, $P = \text{NS}$)(Figure 3C), nor did it provide statistically significant reduction in overall lesion disease burden (Figure 3B). Anti-H3 treatment post-infection did provide some reduction in lesions, though this trend was not statistically significant (Figure 3A, B). These data in combination with the pre-exposure data of Figure 2 indicate that neutralization of VACV MV virions by H3 is more effective at the time of initial exposure to virus than after initiation of infection. This is likely due to lack of neutralization of the EV form.

Protective efficacy of murine anti-VACV mAb combination therapy

Given that anti-B5 exhibited good efficacy and anti-H3 exhibited some efficacy in post-exposure treatment, a combination of anti-B5 and anti-H3 was then tested. This combination neutralizes both EV and MV virions and is the proposed regimen for humans [6]. Mice treated with anti-B5 mAb alone post-exposure were again partially protected from disease, observed by reduction in peak skin lesion size ($P < 0.05$, each timepoint days 4 to 7)(Fig. 4A), delayed onset of disease ($P = 0.01$)(Fig. 4C), and overall lesion disease burden reduction ($P < 0.05$)(Fig. 4B). Mice treated with the anti-B5/H3 combination were in much better health than mice treated with anti-B5 alone. Greatly reduced lesion sizes ($P \leq 0.01$, days 4 to 14)(Fig. 4A) and overall lesion disease burden was observed in mice receiving the anti-B5/H3 combination ($P < 0.01$ vs. control; $P < 0.05$ vs. anti-B5 mAb alone)(Fig. 4B). Most strikingly, disease was completely prevented in approximately half of the animals receiving the anti-B5/H3 mAb combination (Fig. 4C). This was somewhat unexpected, given the earlier poor efficacy of anti-H3 mAb single therapy post-exposure (Fig. 3). Together these data (Fig. 3, 4) indicate that targeting both an EV and MV neutralization target results in improved efficacy in vivo.

Protective efficacy of human anti-VACV mAbs

Given the successful activities of murine anti-B5 and anti-H3 mAbs against VACV in vitro and in vivo in earlier studies [6,18] and against eczema vaccinatum (Fig. 2–4), we developed fully human anti-B5 and anti-H3 mAbs as potential therapeutics [6,7]. Using the eczema vaccinatum model, mice were treated with mAbs after initiation of intradermal VACV infection. A single inoculation of human anti-B5 mAb was provided alone or in combination with a human anti-H3 mAb. Mice that received post-exposure treatment with human anti-B5 mAb alone were partially protected from disease, observed by reduction in peak skin lesion size ($P < 0.05$, day 4)(Fig. 5A–B), delayed onset of disease ($P < 0.05$)(Fig. 5A), lower disease incidence (Fig. 5C), and overall lesion disease burden reduction ($P < 0.01$)(Fig. 5B). Mice treated with the human anti-B5/H3 mAb combination exhibited protection. Greatly reduced lesion sizes ($P \leq 0.01$, days 2 to 12)(Fig. 5A) and overall lesion disease burden was observed in mice receiving the anti-B5/H3 combination ($P < 0.001$ vs. control; $P < 0.01$ vs. anti-B5 mAb alone)(Fig. 5A). Disease was completely prevented in approximately half of the animals receiving the anti-B5/H3 mAb combination ($P < 0.01$)(Fig. 5C). Together these data recapitulate our murine mAb findings shown in Figure 4 that targeting both an EV and MV neutralization target results in increased protection in vivo.

Discussion

Our primary goal here was preclinical validation of a post-exposure prophylactic human anti-MV and anti-EV mAb combination treatment that would be a replacement for VIG as treatment for eczema vaccinatum and other severe side effects of vaccinia immunization, and potentially for treatment of smallpox infection itself. The rationale for this approach is

that mAbs can have much higher specific activity than polyclonal Abs, and recombinant human mAbs can be manufactured in virtually unlimited quantities, under well defined conditions. Here we show that neutralizing mAbs against VACV can be highly efficacious at ameliorating or eliminating eczema vaccinatum in a small animal model. Eczema vaccinatum is associated with poor NK cell activity, and high levels of IL-17, resulting in vastly increased viral loads and progressive skin lesion development [21]. Our data indicate that reduction in the viral load by neutralizing antibodies offsets the defective antiviral activity of the NK cells, likely resulting in clearance of the infection by normal innate and adaptive mechanisms of the immune system, independent of NK cells. Reduction in viral load by these neutralizing antibodies would therefore be likely to be effective at reducing or eliminating clinical disease in people with eczema vaccinatum or other severe side effects of the smallpox vaccine.

We speculate that the combination of anti-H3 and anti-B5 mAbs provides increased protection in this eczema vaccinatum model, and in an immunodeficient mouse model, via several mechanisms of action. The anti-H3 mAb neutralizes MV virions [6], which are released from dead cells in abundance. The anti-B5 mAb neutralizes the other virus form, EV virions, in a complement dependent manner [7,18]. In addition, anti-B5 mAbs direct the complement-mediated killing of VACV infected cells, due to expression of B5 on the surface of infected cells [7,18]. This is a potent mechanism of eliminating reservoirs of replicating virus. It is also plausible that the anti-H3 mAbs specifically enhance the function of anti-B5. Anti-B5 mAbs can direct killing of VACV infected cells, but that then can release a bolus of MV virions, and the presence of anti-H3 mAbs eliminates that problem. In addition, the outer membrane of EV particles is notoriously fragile [22–25], and binding of anti-B5 mAb to EV virions may, in some cases, disrupt the EV membrane and release the infectious MV particle inside. Again, the presence of anti-H3 mAb compensates for this problem by simultaneously neutralizing the MV.

Given the efficacy of these mAbs in three animal models—eczema vaccinatum, respiratory VACV [7], and immunodeficient SCID mice [6]—and their enhanced efficacy in comparison to VIG [6], these human mAbs may also be efficacious against smallpox.

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Figure 1. Induction of erosive skin lesions in VACV-infected eczematous mice

(A) Eczematous skin lesions were induced on the shaved back skin of NC/Nga mice by repeated Der f/SEB (D/B) treatments. The mice with eczematous skin lesions and healthy skin mice were intradermally infected with VACV at the back skin. (B) Typical eczematous of skin (right) and normal skin (left) mice. (C) Typical erosive skin lesions of Western Reserve strain (left panel) and ACAM2000 strain (right panel) of VACV infected mice. Arrows show the sites of VACV infection. Eczematous mice with a clinical score of ≥ 7 were used in the following experiments.

Figure 2. In vivo protection of murine anti-B5 and anti-H3 mAbs injected before VACV infection

(A–D) Eczematous mice were intraperitoneally injected with 200 μ g of anti-B5 or anti-H3 mAb 24 h before intradermal infection of 1×10^6 PFU of VACV. PBS was used as control. (A) Development of the skin lesion was observed for 2 weeks and the size of erosive skin lesion was measured. (B) The disease burden calculated from skin lesion size. ***, $P < 0.001$ vs. control treated mice. (C) Percentage of mice with skin lesions. (D) The change of body weight after VACV infection. Data represent mean and SEM. $N = 10$ –16 each group and the combined data of 3 independent experiments are shown here.

Figure 3. In vivo protection of murine anti-B5 and anti-H3 mAbs injected after VACV infection (A–C) Eczematous mice were intravenously injected with 200 μ g of anti-B5 or anti-H3 mAb 12 h after intradermal infection of 1×10^6 PFU of VACV. PBS was used as control treatment. **(A)** Development of erosive skin lesions was observed for 2 weeks. Data represent mean and SEM values. **(B)** The disease burden calculated from skin lesion size. *, $P < 0.05$ vs. control treated mice. **(C)** Percentage of mice with skin lesions. The data represent one of three experiments. $N = 4$ –7 per group per each experiment.

Figure 4. The effect of combined injection of murine anti-B5 and anti-H3 mAbs for development of skin lesions

(A–C) Eczematous mice were intravenously injected with 200 μ g of anti-B5 or a combination of each 200 μ g of anti-B5 and anti-H3 mAb 12 h after intradermal infection of 1×10^6 PFU of VACV with PBS as control. (A) Erosive skin lesion kinetics. (B) The disease burden calculated from skin lesion size. *, **: $P < 0.05$, $P < 0.01$. The data represent one of two experiments. $N = 5$ –6 per group per each experiment. (C) Percentage of mice with skin lesions. Data are the composite of two experiments. $N = 10$ –12 per group.

Figure 5. Post-infection treatment with human anti-B5 and anti-H3 mAbs

Eczematous mice were intravenously injected with 200 μ g of anti-B5 or a combination of each 200 μ g of anti-B5 and anti-H3 mAb 12 h after intradermal infection of 1×10^6 pfu of VACV with human anti-DNP IgG1 as control. **(A)** Skin lesion size. **(B)** The disease burden calculated from skin lesion size. *, **: $P < 0.05$, $P < 0.01$. The data represent one of two experiments. $N = 5-6$ per group per each experiment. **(C)** Percentage of mice with skin lesions. Data are the composite of two experiments. $N = 10-12$ per group.