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Intranasal cowpox virus infection of the mouse as a model for preclinical evaluation of smallpox vaccines

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Abstract

The intranasal infection of mice with cowpox virus (CPXV) has been evaluated as a model for smallpox infection in man. Administration of a lethal dose of CPXV allowed time for development of T-cell responses but antibodies could not be detected before death occurred. In contrast, infection with a sublethal dose was associated with an early T-cell response followed by neutralising antibodies which correlated with virus clearance. Comparison of two first generation smallpox vaccines revealed no significant differences in terms of immunogenicity, protection and post-challenge virus clearance. These studies show that the CPXV/mouse model is valuable for the initial assessment of smallpox vaccines.

Keywords: Mouse model; Smallpox vaccine; Cowpox virus

1. Introduction

Until 1980, when the World Health Assembly declared its eradication [1,2], smallpox was one of the most dreaded infectious diseases of mankind [3]. Eradication was quickly followed by the cessation of smallpox vaccination in particular because it was sometimes the cause of severe adverse reactions such as eczema vaccinatum, encephalitis and progressive vaccinia [4–6]. Today, the majority of the world's population is no longer immunised [2,7] and therefore would not be protected against a bioterrorist attack using variola virus [8,9].

The smallpox threat strongly argues for the stockpiling of vaccines in order to be prepared to protect entire national populations. In France, health authorities decided to use *vaccinia virus* (VACV) stocks (from calf lymph) produced in the past by the Pourquier Institute but which no longer had an authorization for marketing and to add supplementary doses provided by the Sanofi-Pasteur Company. This company had produced a batch of VACV first generation vaccine.

Because the protective effect of smallpox vaccines cannot be evaluated in man it is important to have pertinent animal models. These vaccines should be assessed for their ability to prevent viral replication and induce humoral as well as cell-mediated immune responses. The European Agency for the Evaluation of Medicinal Products (EMEA) has recommended that vaccines should demonstrate protection against two orthopoxvirus pathogens distinct from VACV and that two different mammalian species be employed starting with a non-primate model [10]. The primary endpoint should be protection against a respiratory challenge.

A number of animal models for smallpox infection have been established such as intranasal (i.n.) administration of VACV or *cowpox virus* (CPXV) Brighton strain to BALB/c mice [11–13], footpad infection with *ectromelia*

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virus (ECTV) [14], aerosolized or intratracheal [15,16] or intravenous [17] *monkeypox virus* infection of Cynomolgus monkeys. However, only a few of these models satisfy EMEA recommendations. With regard to non-primate models, ECTV would not be appropriate because of footpad administration and an i.n. VACV challenge does not demonstrate cross-protection. The intranasal CPXV mouse model [13] appears to be a relevant candidate particularly as it has been shown that i.n. delivery is as efficient an infection route as aerosolisation [13,18]. However, in contrast to the ECTV and VACV models, our understanding of the CPXV model is still insufficient. In particular, the cellular and humoral immune responses which may participate in protection against a primary infection have not been studied although the CPXV model has been used for evaluation of antiviral compounds [12,13,18,19].

Here we report the conditions for sublethal and lethal infection of BALB/c mice with CPXV and describe the major features of viral pathogenesis (replication in the lung and spleen). Furthermore, we have assayed the humoral and cell-mediated immune responses in infected animals and correlated the findings with morbidity and mortality of infected mice. Finally, we have used this model to compare the Sanofi-Pasteur vaccine to the Pourquier Institute vaccine.

2. Materials and methods

2.1. Experimental animals

Four-weeks-old female BALB/c mice (haplotype H-2^d) were purchased from Charles River Laboratories and housed in filter-top micro isolator cages in a biosafety level 3 containment area of the *Centre de Recherches du Service de Santé des Armées* (CRSSA). For pathogenesis studies, mice were infected with CPXV when they were 6 weeks old. For vaccine studies, they were immunised when they were 4 weeks old and challenged 28 or 150 days later. Each assay was performed with six mice per condition. All experiments were approved by the local ethical committee (protocol number: 2002/20 and 2004/37.0).

2.2. Viruses and cells

The Brighton strain of CPXV (ATCC VR 302) was produced in Vero cells (ATCC CCL-81) which were propagated in M199 medium with 5% heat inactivated foetal calf serum (FCS) at 37 °C in a 5% CO_2 atmosphere. P815 cells (ATCC TIB 64, haplotype H-2^d) were cultured in RPMI 1640, supplemented with 10% heat inactivated FCS. Two vaccines (VACV, strain Lister) were used. The lyophilised smallpox vaccine from the Pourquier Institute (batch 986 supplied by the French Department of Defence) was resuspended in its specific glycerine solvent. The liquid smallpox vaccine (Batch number X 5533-1) was supplied by the Sanofi-Pasteur Company (Lyon, France). The titre of the two vaccines was 10^8 pfu/mL.

2.3. Viral dose and inoculation methods

Intranasal infection was performed as previously published [12]. The animals were anesthetised by i.p. injection of ketamine (75 mg/kg) before i.n. administration, in 50 μL, of an adequate dose of CPXV or M199 medium for mock infected mice. For vaccination studies, mice were anesthetized as described above and scarified at the base of the tail with the tip of a 23-gauge needle. Three microliters of vaccine (10^8 pfu/mL) were inoculated at the scarified site. Mice were considered efficiently vaccinated if a take with a characteristic pustule was observed at days 7–10 after inoculation.

2.4. Virus quantification in different organs

Lungs and spleens from mice were crushed with 1 mL per organ of RPMI 1640 containing 0.4% FCS. Lungs, spleens and whole blood suspensions were freeze/thawed three times before centrifugation. Supernatant fluids were used to determine the virus titre (pfu/mL) in Vero cell cultures and the genomic titre by real-time PCR using Taqman chemistry. Genomic quantification was performed using CPXV quantitative PCR on the 14 kDa fusion protein gene (open reading frame A27L) with primers CRSSA 1F (5′-CCTCAGCTTTAACAATTGCTTC-3′) and CRSSA 1R (5′-CCAGCGACTGAATTTTTCTCTACA-3′) hybridized at positions 174–196 and 227–250, respectively and probe CRSSA OPX1 (5′-CGTTTAGCCT-CTGGCTTCTTAGCAGCCT-3′) at position 198–225. Serial 10-fold dilutions of a positive control plasmid were used to derive a standard curve for quantification.

2.5. Antibody assays

The neutralisation titres were determined as previously described [20]. Dilutions of heat-inactivated serum were added to an equal volume of VACV suspension then inoculated on Vero cell monolayers. The antibody neutralisation (NAb) titre was calculated by determining the reciprocal dilution of serum that caused a 50% reduction in the VACV plaque count compared with a negative control.

IgGs specific for CPXV were detected by an indirect ELISA. Plates were coated overnight with CPXV ($10^{4.4}$ pfu/mL previously inactivated with 0.02% formaldehyde). Serial dilutions of serum samples were then added and incubated 1 h at 37 °C. Anti-mouse IgG-peroxidase (Sigma–Aldrich) was added for 1 h at 37 °C. The tetramethyl benzidine reaction was then carried out for 30 min at room temperature and was stopped by 2 N chlorhydric acid. The absorbance was recorded at 450 nm. Titres were calculated as the reciprocal of the highest dilution giving an absorbance at least three-fold above the mean value of 10 negative control wells with serum from uninfected mice.