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Viral vectored immunocontraception: Screening of multiple fertility antigens using murine cytomegalovirus as a vaccine vector

Alec J. Redwood [a,*], Nicole L. Harvey [a], Megan Lloyd [a], Malcolm A. Lawson [a], Christopher M. Hardy [b], Geoffrey R. Shellam [a]

[a] *Microbiology and Immunology, School of Biomedical, Biomolecular & Chemical Sciences, M502, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia*
[b] *Invasive Animals Cooperative Research Centre, CSIRO Entomology, GPO 1700, Canberra ACT 2601, Australia*

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Abstract

Mouse cytomegalovirus (MCMV) has previously been used as a vaccine vector for viral vectored immunocontraception (VVIC). MCMV expressing murine zona pellucida 3 (mZP3) induces long term infertility in up to 100% of female BALB/c mice following a single inoculation. Whilst a large number of antigens have been investigated as potential immunocontraceptive vaccines, it has been difficult to compare these antigens as few studies have used identical approaches or even animal species. Here a range of protein and polyepitope antigens, all expressed by MCMV, were tested for the ability to sterilise female mice. The antigens tested were bone morphogenic protein 15 (BMP15), oviduct glycoprotein (OGP) and ubiquitin-tagged mZP3. In addition, four polyepitope constructs that contain rodent or mouse specific epitopes were tested. This study found that when expressed by an MCMV vector, only full-length mZP3 or ubiquitin-tagged mZP3 induced infertility in female mice. BMP15 and OGP had no effect. Of the four polyepitopes tested, one had a partial effect on fertility. These data indicate that while MCMV is an effective vector for VVIC, the antigen used needs to be tested empirically. The partial infertility seen in mice infected with one of the polyepitope vaccines is a promising finding suggesting that it may be possible to combine a species specific virus with a species specific antigen for use as a disseminating mouse control agent.

Keywords: Cytomegalovirus; Zona pellucida; Polyepitope; Bone morphogenic protein 15; Oviduct glycoprotein

1. Introduction

A wide range of viruses have been identified as potential vaccine vectors, including poliovirus [1,2], vaccinia virus [3], canarypox [4,5], rabies virus [6], adenovirus [7] and herpesviruses [8,9]. Viral vectors have been used to vaccinate against infectious agents [6,7], malignancies [2,5] and induce immunocontraception [10–13]. Viral vectors deliver the antigen directly into host cells, allowing high-level intracellular expression. In addition, because the immune system has evolved a sophisticated array of mechanisms to detect and respond to the antigens of invading viruses they may serve as an adjuvant for the expressed antigen.

We have previously used the herpesvirus, murine cytomegalovirus (MCMV) as a vaccine vector and have reported its use as a viral vectored immunocontraceptive (VVIC) [11,12]. CMVs are members of the β-herpesvirinae of the Herpesviridae and contain a large double stranded DNA genome of approximately 230 Kb [14]. CMVs are found in a broad range of mammalian species but exhibit strict species specificity [15]. They cause asymptomatic infections in immunocompetent animals and persist in a latent state for the lifetime of the host. Their properties of latency, large DNA size and strict species specificity make CMVs useful vac-

* Corresponding author at: Microbiology and Immunology, School of Biomedical, Biomolecular & Chemical Sciences, M502, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia. Tel.: +61 8 9346 2512; fax: +61 8 9346 2912.
E-mail address: aredwood@cyllene.uwa.edu.au (A.J. Redwood).

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cine vectors. CMVs induce strong and long-lived cytotoxic T lymphocyte (CTL) responses [16,17], as well as long-lived antibody responses [18]. It is likely that the persistence and reactivation of MCMV contributes to the long-lived immune responses to MCMV. The ability to clone herpesviruses [19,20], including cytomegaloviruses, as bacterial artificial chromosomes has added to their vaccine potential [12,21].

The success of any vaccination process is dependent not only on the delivery system, in this case the viral vector, but also on the antigen that is delivered. For immunocontraception, the choice of antigen is of particular relevance as the aim of this immunization protocol is to break self-tolerance. This may be easier with some self-antigens than others, as autoantigens are generally restricted to a limited range of proteins [22]. However, breaking self-tolerance may not be sufficient for immunocontraception, as autoimmune responses are not necessarily linked to altered physiology [22]. Consequently, for immunocontraception it is necessary to break tolerance, and for the response to be sufficient in quantity or quality to result in infertility. This could be in the production of an inflammatory response that inhibits normal function in an important organ, such as the ovary or could be due to complete or partial loss of an antigen that is essential for reproduction [23,24], such as zona pellucida 3 (ZP3). Given these complexities it is not possible to determine, except empirically, which antigens will make a suitable target for immunocontraception. Consequently, we chose to test a range of antigens associated with female reproductive processes to determine if immune responses to one or more were capable of sterilising female mice.

Full-length protein antigens were tested as alternatives or improvements on the previously successful mZP3 antigen [11,12]. Possible alternative proteins tested were BMP15 and OGP, both of which are expressed within the reproductive system of female mice [25–27]. The mZP3 antigen was N-terminal ubiquitinated and tested as an improvement on mZ3 alone. Ubiquitination has been shown to improve $CD8^+$ T cell responses and MHC class I antigen processing by targeting proteins to the proteosome [28,29]. Finally, polyepitope antigens were tested in the MCMV vector for the capacity to sterilise female mice. These polyepitope antigens have been previously assessed as immunocontraceptive vaccines when co-administered with adjuvant [30,31] and were included in this study as they contain mouse or rodent specific epitopes. The expression of these polyepitopes by MCMV may enhance the safety of VVIC for widespread use [32] by combining a species specific vector, MCMV, with a species specific antigen.

To allow comparison between the antigens tested, the same MCMV vector, K181, was used for each antigen with the same insertion site, the non-essential *ie2* gene [33]. The expression of each antigen was driven by the HCMV *ie1* (UL123) gene promoter. All infection conditions were identical. We find that the capacity to induce infertility is dependent on the antigen chosen, and that of those tested, only those expressing mZP3, a ubiquitin-tagged mZP3 or the polyepitope B affected the fertility of female BALB/c mice. No demonstrable effect on the fertility of female mice was seen with the viruses expressing mRNA for BMP15, OGP or the polyepitopes A, C or Z.

2. Materials and methods

2.1. Virus and cells

The origins of the K181 strain of MCMV have been described previously [34]. The virus $RM427^+$ was kindly provided by Professor E. Mocarski (Stanford University, Stanford, USA). Virus stocks were propagated in mouse embryonic fibroblasts (MEF) as previously described [35]. Viral titres were determined in duplicate by plaque assay in MEF [11].

2.2. Animals

Specific pathogen-free BALB/c mice were obtained from the Animal Resource Centre (Murdoch, Western Australia) and housed under minimal disease conditions. Mouse care was based on the Australian Code of Practice and was approved by the University of Western Australia Animal Experimentation and Ethics Committee. Sentinel animals were found to be free of a suite of murine pathogens including MCMV following routine testing.

2.3. RNA purification and PCR conditions

Total RNA was prepared as previously described [36] from mouse testes, ovaries or oviducts and polyadenylated RNA was isolated using a PolyAttract System III kit (Promega, Madison, Wisconsin, USA) and reverse transcribed into cDNA using a TimeSaver cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden) with either the pd(T12–18) and pd(N6) primers provided in the kit or gene specific primers. PCR was conducted in 50–100 μl total volumes containing 1–5 ng of DNA, 1–2 μM of each primer, 200 μM of dNTPs, and either 1 unit of *Pfu* (Stratagene, La Jolla, CA) or 2.5 units of *Taq* (Promega) DNA polymerase added "hot start" in the reaction buffers provided by the manufacturers. Nested PCRs were conducted using 1 μl of the first PCR reaction as template.

2.4. Cloning of mouse BMP15 and OGP

Mouse BMP15 (GenBank accession number AF082348) was obtained by nested PCR on BALB/c ovary cDNA reverse transcribed with pd(T12–18) primers. Primers B15F1, B15R1 and B15F2, B15R2 (Table 1) were used for the first and second nested PCRs, respectively. The resulting 1220 bp PCR product was cloned into pGEM-T-easy (Promega, Madison, Wisconsin, USA) to produce plasmid pCMH264. Mouse OGP (GenBank accession number AY521455) was obtained