Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine

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Modified virus Ankara (MVA) is a vaccinia virus (VV) strain that was attenuated by serial passage through chick embryo fibroblasts (CEF) and contains six large genomic deletions compared with parental virus. MVA replicates well in CEFs, but poorly in most mammalian cells. Recombinant MVA is a promising human vaccine candidate due to its restricted host range, immunogenicity and avirulence in animal models, and excellent safety record as a smallpox vaccine. Here we present a further characterization of MVA and demonstrate that: (i) MVA can replicate, albeit poorly, in transformed human cell lines, but not in primary human fibroblasts although there is limited cell-to-cell spread; (ii) MVA is a potent inducer of type I interferon (IFN) from primary human cells, which may restrict virus spread in vivo; and (iii) unlike other VV strains, MVA does not express soluble receptors for IFN-γ, IFN-α/β, tumour necrosis factor and CC chemokines, but does express a soluble interleukin-1β receptor. This provides a plausible and testable explanation for the good immunogenicity of MVA despite its poor replication in mammals. The implications of these findings for the use of MVA as a safe and immunogenic human vaccine candidate are discussed.

Introduction

Recombinant poxviruses have been proposed as new vaccines. Concern about the safety of conventional smallpox vaccines has prompted the development of attenuated derivatives, avian poxvirus vectors, or the use of existing attenuated vaccinia virus (VV) strains such as modified virus Ankara (MVA). MVA was derived by > 500 passages in chick embryo fibroblasts (CEF) of material derived from a pox lesion on a horse in Ankara, Turkey (Hochstein-Mintzel et al., 1972; Mayr et al., 1975). It is highly attenuated yet induces protective immunity against veterinary orthopoxvirus infections (Mayr, 1976; Munz et al., 1993). MVA was used in the final stages of the smallpox eradication campaign, being administered by intracutaneous, subcutaneous and intramuscular routes to more than 120,000 humans in Southern Germany and Turkey. No significant side effects were recorded, despite the deliberate vaccination of high risk groups such as young, old or eczematous patients (Stickl et al., 1974; Mayr et al., 1978; Mahnel & Mayr, 1994). This safety in man is consistent with the avirulence of MVA in animal models, including neonatal and irradiated mice (Mayr et al., 1978).

MVA has genomic deletions totalling 31 kb (Altenburger et al., 1989; Meyer et al., 1991) and produces small white pocks, rather than the large and ulcerated pocks produced by the parental virus cutaneous virus Ankara (Mayr et al., 1975).

Infection of most non-avian cells is abortive (Mayr et al., 1978), partly due to deletions affecting host range genes K1L and C7L. However, some virus replication still occurs on human TK − 143B cells and African green monkey (CV-1) cells (Altenburger et al., 1989) and restoration of the K1L open reading frame (ORF) only partially restores MVA host range (Meyer et al., 1991; Sutter et al., 1994a). Virus replication aborts late during infection of HeLa cells after immature virions are formed (Sutter & Moss, 1992), and this allows efficient expression of proteins by MVA even during abortive infection (Sutter & Moss, 1992; Wyatt et al., 1995). Recombinant MVA expressing influenza virus nucleoprotein and haemagglutinin protected mice against a lethal challenge with influenza virus (Sutter et al., 1994b). Likewise, recombinant MVA expressing simian immunodeficiency virus (SIV) antigens provided re-
sistance to SIV-induced disease and afforded better protection than VV strain Wyeth (a current human vaccine) expressing the same SIV proteins (Hirsch et al., 1995, 1996). Recombinant MVA also has potential as a tumour vaccine (Carroll et al., 1997). In view of its safety and immunogenicity, recombinant MVA is considered a promising human vaccine candidate (Moss et al., 1996).

Orthopoxviruses have evolved strategies for evasion of the host immune response that include the secretion of receptors for tumour necrosis factor (TNF) (Hu et al., 1994; Smith et al., 1996), interleukin (IL)-1β (Alcamí & Smith, 1992; Spriens et al., 1992), interferon (IFN)-γ (Alcamí & Smith, 1995; Mossman et al., 1995), IFN-α/β (Colamonici et al., 1995; Symons et al., 1995) and CC chemokines (Graham et al., 1997; C. A. Smith et al., 1997; Alcamí et al., 1998). These soluble receptors generally inhibit the host response, and in most cases their presence is associated with increased virulence. The IL-1β receptor (IL-1βR) is an exception: when virus was administered to mice by the intranasal route the IL-1βR diminished the signs of illness and weight loss, and prevented fever (Alcamí & Smith, 1992, 1996).

Here we report that MVA lacks functional receptors for TNF, IFN-γ, IFN-α/β and CC chemokines, but expresses the IL-1βR. In addition, we show that MVA undergoes limited replication in transformed human cells such as TK−143B and HeLa cells, but not in primary human fibroblasts, although there is some evidence of cell-to-cell spread on immunostaining. In primary human cells MVA induces release of type I IFN. The significance of these results for the development of MVA as a human vaccine is discussed.

Methods

**Cells and viruses.** Human MRC-5, TK−143B, HeLa and FS-2 cells (primary human foreskin fibroblasts) were prepared by either the cell bank or P. Handford, Sir William Dunn School of Pathology, University of Oxford, UK. CEFs were prepared from whole embryo digests and grown in minimal essential medium (MEM) with 10% foetal bovine serum (FBS). Confluent monolayers of CEF were maintained at 31 °C for up to 4 weeks, with weekly changes of medium (MEM with 2% FBS). Fresh CEF monolayers were prepared the day before use by 1:3 split and overnight growth in MEM with 10% FBS.

The sources of VV strains Western Reserve (WR) and Lister and cowpox virus (CPV) strain Brighton Red and cocal virus have been described (Alcamí & Smith, 1995). MVA from human vaccine stock (II/85) and from passage 575 was kindly provided by A. Mayr, Veterinary Faculty, University of Munich, Munich, Germany. Unless otherwise stated, experiments relate to MVA II/85. MVA was routinely propagated and titrated in CEF grown in MEM. Recombinant baculovirus expressing the VV IL-1βR (AcB18R) or the VV IFN-α/β receptor (IFN-α/βR) (AcB18R) have been described (Alcamí & Smith, 1992).

**Immunostaining.** FS-2 cells were infected with MVA at 0·001 p.f.u. per cell and foci were stained 72 h post-infection (p.i.) with an anti-VV serum raised by live infection of a rabbit. Bound antibody was detected by peroxidase-conjugated polyclonal goat anti-rabbit (Sigma) then developed with diaminobenzidine (Sigma) in the presence of nickel sulphate and hydrogen peroxide.

**Cytokines.** Human recombinant 125I-IL-1β (80 µCi/µg), 125I-TNF-α (80 µCi/µg), 125I-IFN-γ (90 µCi/µg), 125I-RANTES (2200 Ci/mmole) and 125I-MIP-1α (2200 Ci/mmole) were obtained from Du Pont–New England Nuclear. Human recombinant IFN-γ (1 × 10^6 units/mg) was obtained from Genzyme and human natural IFN-α (1.5 × 10^6 units/mg) was obtained from Wellcome. Human recombinant IL-1β (2 × 10^6 units/mg) and TNF-α (2 × 10^6 units/mg) were from R&D Systems, and IL-1α (8 × 10^6 units/mg) was from Genzyme.

**Preparation of medium for binding and biological assays.** Unless otherwise stated, supernatants were harvested from CEFs 18 h.p.i. with MVA at 5 p.f.u. per cell, or from TK−143B cells or HeLa cells 48 h.p.i. with CPV, VV strain WR or Lister at 10 p.f.u. per cell and were prepared as described (Alcamí & Smith, 1992). VV supernatants were inactivated with 4.5,8-trimethylpsoralen and ultraviolet light for biological assays for IFN (Tsung et al., 1996). Supernatants from insect cultures infected with recombinant baculovirus were prepared as described (Alcamí & Smith, 1992).

**Binding assays for cytokines, chemokines and interferons.** Soluble binding assays for IL-1βR and TNFR were performed by incubating 100 pM of 125I-IL-1β or 125I-TNF with culture supernatants and precipitation of the ligand–receptor complexes with polyethylene glycol and filtration through Whatman GF/C filters as described (Alcamí & Smith, 1992). Background radioactivity precipitated in the presence of medium was subtracted. Cross-linking experiments with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to 125I-IFN-γ (2 nM), 125I-RANTES (0.4 nM) or 125I-MIP-1α (0.4 nM) were performed in 20 µl as described (Alcamí & Smith, 1995; Alcamí et al., 1998). Samples were analysed by electrophoresis in 14% polyacrylamide gels.

**Activity assay for IFN.** The biological activity of human IFN was assayed by its ability to inhibit plaque formation by cocal virus (a rhadovirus related to vesicular stomatitis virus) in cultures of HeLa cells as described (Alcamí & Smith, 1995). Cell monolayers were pre-treated for 16–24 h with IFN and virus-free supernatants, infected with approximately 100 p.f.u. of cocal virus, and plaques counted 48 h.p.i.

**Cloning and sequencing of B8R gene and flanking regions.** The B8R gene and flanking regions were amplified from MVA DNA by PCR using Pyroccocus furiosis polymerase and the primers 5′-CTAGAATTCAACGGCAGTCTACACG 3′ and 5′-TCAAGACTTCGTCAGTGGG 3′. The products of four separate PCR reactions were cloned into pCR-Script (Stratagene) and sequenced on both strands.

**Results**

MVA has a restricted replication in mammalian cells, yet is a potent immunogen inducing immune responses equal to or greater than those induced by fully replication competent VV strains such as WR or Wyeth. Since many orthopoxviruses secrete proteins from infected cells that interfere with the host response to infection and which might diminish virus immunogenicity, we wondered if the enhanced immunogenicity of MVA reflected the loss of these immunomodulatory proteins. To assess this we performed binding assays or biological assays for receptors for IL-1β, TNF, IFN-α/β, IFN-γ and CC chemokines in the supernatant of cells infected with MVA.

**Cytokine receptor profile of MVA**

To determine if MVA expressed an IL-1βR, supernatants from mock- or virus-infected CEFs were tested in a soluble
binding assay with human $^{125}$I-IL-1$\beta$. Fig. 1(a) shows that CEFs infected with MVA secrete an IL-1$\beta$ binding activity that could be detected in the supernatant from only 200 cells. An IL-1$\beta$R was produced by HeLa cells infected with VV strain WR, but also by the non-replicating MVA. As the B15R gene shown to express a soluble chemokine binding protein (CKBP) (Alcamí et al., 1997) reported that the protein bound CC and CXC chemokines, while using biological assays we showed a specificity for only CC chemokines (Alcamí et al., 1998). To determine if MVA expressed a CC CKBP, human $^{125}$I-labelled RANTES or MIP-1$\alpha$ was incubated with the supernatants of MVA-infected CEFs in the presence of a chemical cross-linker, followed by electrophoresis and autoradiography (Fig. 2). Cells infected with VV strain Lister or CPV expressed a CKBP which bound both these human CC chemokines and was detected with only 1 $\mu$l of supernatant, and as previously noted the size of the CPV protein was smaller than that from VV Lister (Alcamí et al., 1998). In contrast, no CC chemokine binding activity was detected in 20 $\mu$l of supernatant of mock-infected cells or cells infected with VV WR, MVA 575 or MVA II/85.

**MVA lacks IFN-$\gamma$ and IFN-$\alpha/\beta$ receptors**

Most VV strains express a type I and type II IFNR (Alcamí & Smith, 1995; Symons et al., 1995). The presence of these proteins in MVA-infected cell supernatants was therefore examined by cross-linking with human $^{125}$I-IFN-$\gamma$ (Fig. 2) and bioassays for type I and type II IFNs (Fig. 3). The supernatants from cells infected with CPV and VV WR and Lister each expressed a protein which bound $^{125}$I-IFN-$\gamma$ (Fig. 2) and, as previously noted (Alcamí & Smith, 1995), the CPV protein was slightly smaller than that expressed by either VV strain. In contrast, neither MVA isolate expressed an IFN-$\gamma$R. This result was confirmed with a biological assay. Pre-treatment of HeLa cells with human IFN-$\gamma$ or IFN-$\alpha$ inhibits plaque formation by coxal virus. However, the antiviral activity of these IFNs was reversed by pre-incubating IFN with the supernatant from CPV-infected cells (Fig. 3, CPV). CPV is known to express an
Fig. 2. MVA does not express an IFN-γR or a CC CKBP. Supernatants from $4 \times 10^4$ TK$^{-}143$B cells uninfected (mock) or infected with CPV, VV Lister or VV WR or from the same number of CEFs infected with MVA 575 or MVA II/85 were incubated with $^{125}$I-IFN-γ, $^{125}$I-RANTES or $^{125}$I-MIP-1α. Complexes were cross-linked with EDC, resolved from monomeric or dimeric ligands by PAGE and detected by autoradiography. The positions of IFN-γ monomers (M), IFN-γ dimers (D), RANTES (R), MIP-1α (MIP) and ligand receptor complexes (arrowheads) are indicated. Molecular masses in kDa are shown.

Fig. 3. Bioassay for type I and type II IFNRs. HeLa cell monolayers in 24-well plates were infected with approximately 100 p.f.u. of cocal virus in the presence of supernatant from $10^5$ CEFs that had been infected with MVA, or from TK$^{-}143$B cells that had been either mock-infected or infected with CPV. Either 50 U/ml of human IFN-α or 20 U/ml of human IFN-γ or no IFN were included in the overall medium containing 1-5% (w/v) carboxymethylcellulose as indicated. Cells were stained with 0-1% crystal violet in 15% ethanol 48 h p.i. The presence of IFNR activity is demonstrated by reversal of IFN-induced suppression of cocal virus plaque formation.
IFN-γ-R and IFN-α/βR that neutralize these human IFNs (Alcamí & Smith, 1995; Symons et al., 1995). No such inhibitory activity was expressed from MVA-infected CEFs (Fig. 3, MVA) or mock-infected cells.

Although MVA did not express an IFN-γ-R, PCR analysis using primers flanking the VV WR B8R gene amplified an MVA DNA fragment of similar size to that of VV WR (data not shown). The MVA IFN-γ-R locus was therefore sequenced and compared with the VV WR B8R gene (Smith et al., 1991). The sequences were identical except for three silent nucleotide substitutions and three short deletions in MVA. The first two deletions eliminated 24 or 21 nucleotides and therefore retained the ORF while removing 8 or 7 amino acids, respectively (Fig. 4a). The third deletion of 23 nucleotides introduced a frameshift resulting in termination immediately downstream. The two amino acid changes between the B8R proteins in the WR and Copenhagen strains of VV are indicated underneath the WR sequence. (b) Nucleotide sequence alignments for MVA and WR showing the three deletions within the B8R ORF. Short direct oligonucleotide repeats flanking the deletions are underlined. Numbers refer to the nucleotide positions within a 42 kbp region of VV strain WR (Smith et al., 1991).

Fig. 4. B8R sequence comparisons. (a) Alignment of predicted protein sequences for B8R of MVA and VV WR, showing effects of three deletions within the ORF. Note that the third deletion introduces a frameshift resulting in termination immediately downstream. The two amino acid changes between the B8R proteins in the WR and Copenhagen strains of VV are indicated underneath the WR sequence. (b) Nucleotide sequence alignments for MVA and WR showing the three deletions within the B8R ORF. Short direct oligonucleotide repeats flanking the deletions are underlined. Numbers refer to the nucleotide positions within a 42 kbp region of VV strain WR (Smith et al., 1991).

MVA can replicate in transformed but not primary human cells

MVA replicates poorly in most mammalian cells but well in avian cells such as CEFs. In human cells, MVA has been reported to replicate in TK−143B cells (Altenburger et al., 1989) but not in HeLa cells (Meyer et al., 1991; Sutter & Moss, 1992). If recombinant MVAs are to be used for immunoprophylaxis or immunotherapy in humans, it is important to establish whether or not the virus can replicate in human cells. Therefore, we examined MVA replication in four human cell types and compared these to CEFs and mouse BALB3t3 cells (Fig. 5a). The human cells selected were two transformed cell lines (HeLa and TK−143B) and two non-transformed or primary cell cultures (MRC-5 and FS-2). Infections were performed at 0.05, 0.5 and 5 p.f.u. per cell and the data are expressed as the increase or decrease in titre at 48 h p.i. relative to the input virus. At each m.o.i. there was a net increase in virus titre in HeLa and TK−143B cells but a decrease in MRC-5 and FS-2 cells. The increase in titre in TK−143B cells was reproducibly observed. In non-transformed human cells there was a consistent 5- to 10-fold drop in titre. Likewise, infection of mouse BALB3t3 cells consistently yielded less virus than input.

MVA can induce focus formation on FS-2 cells

Although MVA did not increase in titre in FS-2 cells, the fact that the virus titre at 48 h p.i. was only 5-fold lower than the original virus input suggested that some limited virus...
replication might have taken place. Without any virus replication, the titre of virus present at 48 h p.i. would be expected to be orders of magnitude lower due to the uncoating and loss of infectivity of the great majority of the input virus. We therefore assessed whether MVA could spread from the initial infected cell by infecting FS-2 cells at 0·001 p.f.u. per cell and staining with a polyclonal anti-VV serum 3 days later (Fig. 5b). Foci (more than two adjacent staining cells) were detected where VV antigen had spread to surrounding cells, although 98% of infected cells were infected in isolation. This was consistent with virus replication and spread from a limited number of infected FS-2 cells.

**MVA is a potent inducer of type I IFN**

To investigate why MVA was replicating in transformed but not primary human cells, the ability of these cells to produce type I IFN after MVA infection was assessed (Fig. 6). Type I IFN released from these cells would remain active because MVA does not express a type I IFNR (Fig. 3). The presence of IFN in these supernatants was measured by inhibition of plaque formation by cocal virus on HeLa cells. HeLa cells infected by MVA produced no activity that inhibited cocal virus replication in fresh HeLa cells. In contrast, supernatant from MVA-infected TK−143B, FS-2 and MRC-5 cells all contained an inhibitor. This was partly (TK−143B cells) or completely (FS-2 and MRC-5 cells) mediated by type I IFN, because addition of the VV WR soluble IFN-α/βR, but not soluble IL-1βR, expressed from recombinant baculovirus (Alcamí & Smith, 1992; Symons et al., 1995) reversed this inhibition (Fig. 6). The most dramatic result was seen with MRC-5 cells where MVA infection induced sufficient type I IFN to inhibit cocal virus plaques by approximately 90% and this inhibition was completely reversed by the IFN-α/βR. The inhibition was only approximately 50% using supernatants from MVA-infected FS-2 cells, but as for MRC-5 cells, this was completely reversed by the IFN-α/βR. The situation with TK−143B cells was more complex because although MVA-infected TK−143B cell supernatants inhibited
cocal virus formation by approximately 80%, this was only partially reversed by the IFN-α/βR, suggesting another inhibitor was present.

The induction of type I IFN by MVA infection of primary human fibroblasts and the lack of an MVA encoded IFN-α/βR suggested that production of IFN might explain why MVA replication in these primary human fibroblasts was abortive. However, incubation of either MRC-5 or FS-2 cells with VV IFN-α/βR from recombinant baculovirus throughout infection with MVA did not enable an increase in virus titre (data not shown). Thus other factors restrict MVA replication in these cells.

**Discussion**

MVA is a severely attenuated VV strain that has safely prevented orthopoxvirus infections in man and animals. MVA is unable to replicate in most mammalian cell lines, but despite this it is more immunogenic than replication competent vaccinia virus Wyeth in animal models. Consequently, MVA is a very promising candidate antigen delivery vehicle for immunoprophylaxis and immunotherapy that is being developed as an alternative to conventional VV strains or recombinant avipoxviruses. It is important to understand better why MVA is so immunogenic despite its attenuation and to examine further the replicative potential of MVA in human cells. This is especially important given the potential to vaccinate people who may be immunosuppressed due to HIV infection. In this paper we have addressed both these issues and show that MVA (i) lacks several of the immunomodulatory proteins expressed by many other orthopoxviruses, (ii) can replicate poorly in some human cells and (iii) can induce production of type I IFN from primary human fibroblasts.

Following the development of recombinant poxviruses as expression vectors and potential live vaccines in 1982, several laboratories characterized VV virulence genes in order to attenuate the virus and make it a safer vaccine. This work identified many VV proteins that interfere with the host response to infection (for review see G. L. Smith et al., 1997). A group of these proteins function as soluble inhibitors of cytokines, chemokines and IFNs. These prevent their ligands reaching the cellular receptors and triggering the inflammatory and immune response to infection. By studying the roles of these immunomodulatory proteins in VV and other systems, we consider that the presence of the IL-1βR and the absence of receptors for IFN-α/β, IFN-γ, TNF and CC chemokines might represent a suitable profile for a safe and immunogenic orthopoxvirus vaccine. The expression of these proteins in MVA was therefore characterized and revealed that MVA has exactly this profile. Moreover, out of the 16 strains of VV that we examined (buffalopox, Copenhagen, Dairen, Evans, IHD-J, IHD-W, King Institute, Lister, MVA, Patwadangar, rabbitpox, Tashkent, Tian Tan, USSR, WR, Wyeth) MVA is the only strain with such a profile (Alcamí & Smith, 1992, 1995, 1996; Symons et al., 1995; Alcamí et al., 1998; A. Alcamí and others, unpublished data).

The loss of the IFN-γR is rare in VV strains (Alcamí & Smith, 1995), implying that this is an important protein for VV. IFN-γ is important in promoting the Th-1 type response to infection that includes the induction of cytolytic T cells. A virus lacking an IFN-γR is thus likely to be better recognized by the immune system and therefore be less virulent, while at the same time be able to induce a stronger immune response and therefore be more immunogenic. The virulence and immunogenicity of VV strains lacking the IFN-γR have not been reported, but a myxoma virus mutant lacking the IFN-γR is attenuated (Mossman et al., 1996), although this attenuation could also be due to binding of chemokines by this protein (Lalani et al., 1997). Although the lack of an IFN-γR is probably an important factor contributing to the attenuated and immunogenic phenotype of MVA, other immunomodulatory molecules may play a role. This is implied by the demonstration that MVA is severely attenuated in mice (Mayr et al., 1978), a host in which the VV IFN-γR is ineffective (Alcamí & Smith, 1995).

The loss of the IFN-α/βR may also be quite important due to the ability of VMA to induce a type I IFN response, the established role of the IFN-α/βR as a factor promoting VV virulence (Colanomici et al., 1995; Symons et al., 1995), the ability of type I IFN to have direct antiviral activity and to enhance immunological memory (Tough et al., 1996), and the fact that the only other VV strains which lack the IFN-α/βR (Lister) or express a receptor with a greatly reduced affinity for human IFN-α2 (Wyeth) were among the safer smallpox vaccines (Fenner et al., 1988). Lack of a TNFR, which has only been shown to decrease myxoma virus virulence (Upton et al., 1991), is also likely to enhance MVA safety and immunogenicity due to the direct antiviral activity and pro-inflamatory activity of this cytokine. However, it may not explain the enhanced immunogenicity of MVA compared to many other VV strains, since the majority of these strains do not express TNFRs (A. Alcamí and others, unpublished data). The loss of a soluble inhibitor of a broad range of CC chemokines, which induce migration of leukocytes to sites of infection, is also likely to restrict virus replication in infected tissues and possibly enhance its immunogenicity.

The expression of an IL-1βR by MVA is interesting and consistent with the observation that (i) expression of an IL-1βR by other VV strains prevents fever and reduces virulence in mice (Alcamí & Smith, 1992, 1996), and (ii) IL-1βR were produced by the safer smallpox vaccines (Alcamí & Smith, 1992). However, these observations were made in the context of severe experimental infection or human vaccination with fully replication competent VV. It is possible that due to the very limited replication of MVA, the IL-1βR might have little effect upon virulence and possibly suppress the immune response. IL-1β has been reported to increase the primary immune response (Plebanski et al., 1992) and thus deletion of
this gene might enhance the immunogenicity of MVA. Potent immunogenicity of viruses, such as MVA, with severely restricted replication in vaccinees, and thus low level of antigen production, may be necessary for efficacy.

It is quite possible that there are mutations in the MVA genome other than those reported here which influence immunogenicity and host range. The precise roles of the virus immunomodulatory proteins described here will require the re-introduction or deletion of these genes from the MVA genome and direct comparisons of immunogenicity. However, given our knowledge of the function of the ligands bound by these molecules, the virus proteins are excellent candidates to influence the host response to infection.

The limited replication of MVA in some human cells is relevant to its potential use as a human vaccine. The virus titre increased in transformed human cells (TK–143B and HeLa) but decreased after infection of primary human fibroblasts (MRC-5 and FS-2). Even in primary fibroblasts it is possible that infectious particles are produced in some cells because after infection of FS-2 cells at low multiplicity, foci of infection were visible after immunostaining with anti-VV antibody. The fact that only a small proportion (approximately 2%) of infected cells yielded foci suggests that this was a stochastic event caused by virion replication and spread rather than translocation of virus antigen. The inability to replicate well in primary human cells might be attributable to several factors including loss of the human host range gene and production of type I IFN, although replication in human cells was not restored after addition of a soluble inhibitor of human type I IFN. In addition, in HeLa cells MVA shows similar sensitivity to type I IFN as VV strain WR lacking the type I IFNR (data not shown). This suggests that intracellular modulators of IFN such as the E3L and K3L gene products might be expressed by MVA, but further work is required to confirm this. The ability of MVA to induce type I IFN, while not expressing an IFN-\(\alpha/\beta\)R, would be a desirable feature in vivo that would restrict MVA replication and probably enhance vaccine safety and immunogenicity. Importantly, IFN induction would still occur in vaccinees that had an impaired immune system.

In summary, this study provides a further characterization of MVA and shows that this virus can replicate poorly in transformed human cell lines, but not in primary human fibroblasts where it is a potent inducer of type I IFN. Unlike other VV strains, MVA does not express soluble proteins that bind IFN-\(\gamma\), IFN-\(\alpha/\beta\), TNF and CC chemokines, but does express a soluble IL-1\(\beta\) receptor. This provides a plausible explanation for the good immunogenicity of MVA despite its poor replication in mammals. Lastly, the B8R locus is identified as another locus into which foreign antigens or adjuvant molecules may be inserted for expression by recombinant MVAs.

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