In vitro expression and analysis of secreted fowlpox virus CC chemokine-like proteins Fpv060, Fpv061, Fpv116 and Fpv121

A. Jeshtadi1, G. Henriquet1, S. M. Laidlaw1, D. Hot2, Y. Zhang3, and M. A. Skinner1

1Institute for Animal Health, Division of Molecular Biology, Compton, Newbury, Berkshire, U.K.
2Laboratoire des Biopuces, Institut Pasteur de Lille, Lille, France
3Department of Oral Biology, University of Nebraska Medical Center, College of Dentistry, Lincoln, Nebraska, U.S.A.

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Summary. The four CC chemokine-like proteins (Fpv060, Fpv061, Fpv116 and Fpv121) of fowlpox virus (FWPV) were over-expressed as His-tagged versions from a T7 promoter/EMCV IRES construct in vitro, by coupled transcription/translation, or in cell culture, by co-infection with two recombinant FWPVs (one expressing the chemokine-like protein and one expressing T7 RNA polymerase). All, except Fpv116, appeared to be glycosylated in the presence of microsomal membranes in vitro. In culture, all were secreted (even though secretion of Fpv061 was not predicted). Secreted forms of Fpv060 and Fpv121 were the most abundant forms of those two proteins. Glycosidase analysis of cellular and secreted forms confirmed that Fpv060, Fpv061 and Fpv121 were N-glycosylated and that the most abundant, cellular form of Fpv061 had been glycosylated but remained Endo H-sensitive (retained in the endoplasmic reticulum or Golgi). N-terminal sequence analysis of His-tagged Fpv060 and Fpv121 showed that they were processed at the predicted signal cleavage sites. Fpv060- and Fpv061-specific antipeptide sera allowed confirmation that the expression, processing and secretion of the native proteins were as determined for the His-tagged proteins. Isolation of knock-out mutants showed that all four proteins were non-essential for replication in tissue culture.

Introduction

The genome sequences of a pathogenic US strain [1] and attenuated European strain, FP9 [14], of fowlpox virus (FWPV) (Fowlpox virus is the type species of
the genus *Avipoxvirus*, family *Poxviridae*) have been determined. The predicted proteins encoded by some of the open reading frames (ORFs) showed sequence similarity to several classes of immunomodulatory proteins. Thus putative viral chemokines, chemokine receptors, an interleukin 18-binding protein (IL-18BP), β-nerve growth factors, a transforming growth factor beta (TGF-β), natural killer cell receptors, semaphorin and serine proteinase inhibitors were identified [1].

A wide variety of cell types are able to produce and release chemotactic cytokines, known as chemokines, as a host response to allergens, infections or trauma. The chemokines selectively attract leukocytes to the affected tissue resulting in an inflammatory response, which may lead to protection. The chemokines are small proteins, the molecular mass of their primary translation products are mainly about 9 to 12 kDa. On the basis of the conserved arrangement of cysteine (C) residues, the chemokines have been classified into CXC (or alpha) chemokines, CC (or beta) chemokines, C (or gamma) chemokines and CX3C (or delta) chemokines.

Several viral chemokine homologues have been identified and are being characterised. The MC148R protein, a CC chemokine homologue from Molluscum contagiosum virus (MOCV), a human poxvirus, has been shown to exhibit broad-spectrum antagonistic activity against cellular chemokines [6, 7, 12, 22]. In contrast, the herpesvirus CC chemokine-like proteins, encoded by Murine cytomegalovirus (MCK-1 and MCK-2), Human herpesvirus 6 (U83 product) and Human herpesvirus 8 (vMIP-I, v-MIP-II and vMIP-III), all seem to act as agonists, though usually with different receptor specificity to their host equivalents [9, 20, 21, 24, 27].

Unlike MOCV, which encodes only one chemokine-like protein [22], FWPV has four ORFs (fpv060, fpv061, fpv116 and fpv121) with sequence similarity to CC chemokines [1]. Their sequences are conserved in the tissue culture attenuated strain FP9 [14]. At a length of 188 amino acids, however, the protein encoded by fpv060 is longer than the other FWPV chemokine-like proteins (120 to 129 amino acids) and cellular chemokines. MC148R is predicted to be expressed early [23] but only for one of the four FWPV chemokine-like genes (fpv061) has it been possible to predict early expression [1]. Moreover, only three of the four proteins (Fpv060, Fpv116 and Fpv121) were predicted [1], using SigCleave [16], to be secreted. We confirmed, using SignalP-NN and SignalP-HMM [19], that the secretion of Fpv061 was not predicted. The FWPV chemokine-like proteins may play an important role during infection in evading host immune responses but demonstration of specific interactions will prove difficult as sequences encoding chicken chemokines and chemokine receptors are only just being identified so...