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Species Specificity of Ectromelia Virus and Vaccinia Virus Interferon-γ Binding Proteins

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Interferon-γ functions within the immune system as a potent anti-viral and immunoregulatory cytokine. In order to successfully replicate within a host cell, poxviruses have evolved a number of strategies to counteract the pleiotropic effects of interferon-γ. In particular, the leporipoxvirus myxoma virus was shown to express an extracellular soluble interferon-γ receptor homolog, denoted M-T7, which is capable of inhibiting the anti-viral activities of rabbit interferon-γ (C. Upton, K. Mossman, and G. McFadden, 1992, *Science* 258, 1369–1372). Here, we demonstrate that expression of soluble interferon-γ receptor homologs appears to be characteristic of all poxviruses tested, including Shope fibroma virus, vaccinia virus (strains WR and IHDW), ectromelia virus, cowpox virus, and rabbitpox virus. We have cloned, sequenced, and characterized the interferon-γ binding protein in supernatants from ectromelia virus-infected cells, and demonstrate the capability of this soluble protein to bind human, murine, and rabbit interferon-γ with similar affinity. We also investigate the properties of the vaccinia virus interferon-γ binding protein and demonstrate that this protein binds human and rabbit interferon-γ with similar affinity and binds murine interferon-γ with a significantly lower relative affinity. The implications of these studies with respect to viral pathogenesis and the evolutionary relationship between a virus and its host are discussed.

Interferon-γ (IFN-γ) is a pleiotropic cytokine which serves critical functions within the immune system both in the presence and in the absence of pathogenic challenge (Epstein, 1984; Vilcek *et al.*, 1985; Farrar and Schreiber, 1993). IFN-γ exerts its anti-viral and immunoregulatory effects on the immune system through ligand-dependent signaling of the IFN-γ receptor (IFN-γR). The IFN-γR is a complex composed of the α chain, a single membrane-spanning polypeptide responsible for ligand binding and signal transduction (Aguet *et al.*, 1988; Gray *et al.*, 1989), and a β chain which confers functional species specificity to the transduced signal (Soh *et al.*, 1994; Hemmi *et al.*, 1994).

The importance of IFN-γ in the response to viral infection has been amply demonstrated, particularly in the case of poxviruses (Buller and Palumbo, 1991; Karupiah *et al.*, 1993a; Melkova and Esteban, 1994). Poxviruses are characterized as large, double-stranded DNA viruses that replicate within the cytoplasm of host cells (Moss, 1990). In a murine experimental model, infection with the orthopoxvirus vaccinia virus, which is effectively cleared by the immune system, becomes lethal upon addition of monoclonal antibodies to IFN-γ (Ruby and Ramshaw, 1991). Likewise, in athymic nude mice, where vaccinia virus infection is lethal, clearance is attained when infection with a recombinant virus expressing IFN-γ is utilized (Kohonen-Corish *et al.*, 1990; Ramshaw *et al.*, 1992). IFN-γ has also proved critical in the recovery of mice from ectromelia virus, the causative agent of mousepox (Karupiah *et al.*, 1993a).

In response to the potent anti-viral and immunoregulatory effects elaborated by IFN-γ during viral infection, poxviruses have evolved a variety of defense mechanisms to effectively reduce the efficiency of the host immune response. For example, IFN-γ (and especially IFN-α and IFN-β) induction of anti-viral activity through the RNA-dependent protein kinase pathway is blocked at the stage of double-stranded RNA activation and phosphorylation of the initiation factor eIF2-α by vaccinia virus E3L and K3L intracellular proteins, respectively (Chang *et al.*, 1992; Davies *et al.*, 1992). While E3L and K3L block certain intracellular IFN-γ-induced pathways, poxviruses are also capable of inhibiting IFN-γ extracellularly, prior to receptor engagement. Cells infected by the leporipoxvirus myxoma virus were shown to secrete a 37-kDa protein, designated M-T7, which possesses homology to the ligand binding domain of the known mammalian IFN-γRs and effectively functions as a soluble IFN-γR homolog (Upton *et al.*, 1992). Here, we demonstrate that secretion of an IFN-γ binding protein is not unique to myxoma virus, and investigate the properties of IFN-γ binding proteins from ectromelia virus and vaccinia virus.

MATERIALS AND METHODS

Viruses and cells

Ectromelia virus (Moscow strain) (Chen *et al.*, 1992) was cultured in primate BSC-1 cells in Eagle's minimal

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essential medium plus 10% fetal calf serum. vMyxlac, a myxoma virus (strain Lausanne) derivative containing the *Escherichia coli* lacZ marker gene inserted at an innocuous site between the myxoma growth factor and M9 genes (Opgenorth *et al.*, 1992), Shope fibroma virus (SFV), vaccinia virus (strains WR and IHDW), cowpox virus, and rabbitpox virus were cultured in primate BGMK cells, in Dulbecco's minimal essential medium supplemented with 10% newborn calf serum. Cowpox and rabbitpox viruses were a generous gift from Dr. R. Moyer. All cells were supplied by the ATCC.

Cloning and sequencing of the ectromelia virus IFN-γ receptor homolog

The ectromelia virus gene was localized to a 4.5-kb *Bam*HI fragment by Southern blotting using a PCR product of the vaccinia virus B8R gene as probe. Clone EV-B93 was isolated by size from a shotgun library of ectromelia virus *Bam*HI fragments in pBluescript (KS^+) (Stratagene). DNA sequencing of the region of interest was accomplished using Sequenase II (Amersham) with dsDNA template and custom primers. This sequence has been submitted to the GenBank Database (Accession No. U19584).

Generation of cellular supernatants

BSC-1 and BGMK cells were either mock infected or infected with ectromelia virus (BSC-1) or vMyxlac, SFV, vaccinia, cowpox, or rabbitpox viruses (BGMK) at a multiplicity of infection (m.o.i.) of 5–10. After 1 hr at 37°, the monolayers were washed twice to remove unabsorbed virus and resupplemented with serum-free medium for a further 8 (BSC-1) or 24 hr (BGMK). Supernatants were collected, centrifuged at 5000 *g* for 15 min to remove cellular debris and concentrated using Amicon Centriprep-10 filters.

Binding assays of interferon-γ with proteins secreted from poxvirus-infected cells

Purified rabbit, human, and murine IFN-γ (generously supplied by Genentech) were labeled *in vitro* with protein kinase A (Sigma) and [γ-^{32}P]ATP (ICN) as previously described (Kung and Bekesi, 1986), with the exception that 5 μg of IFN-γ was phosphorylated at 30° for 30 min. Chemical cross-linking assays were performed using 10 μl concentrated mock-infected or virus-infected supernatants with 2 ng radiolabeled IFN-γ as previously described (Upton *et al.*, 1992). The resulting protein complexes were analyzed by SDS–PAGE followed by autoradiography. For competition binding assays, cross-linking assays were performed as described above, with simultaneous addition of increasing amounts of unlabeled competitor IFN-γ with the radiolabeled IFN-γ. Unlabeled competitor values of 10, 20, 40, and 80 ng IFN-γ were used.

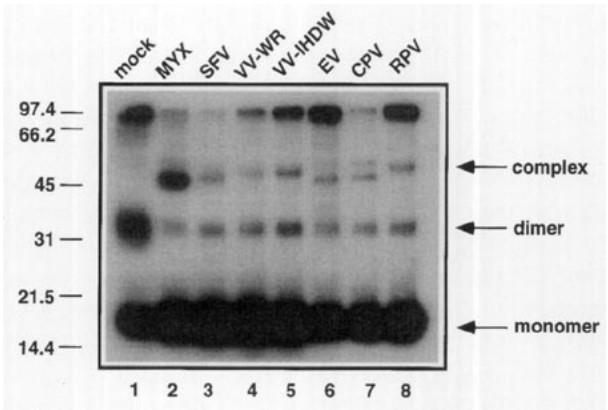

FIG. 1. Poxviruses express secreted IFN-γ binding proteins. Supernatants from mock-, myxoma virus (MYX)-, Shope fibroma virus (SFV)-, vaccinia virus (VV, strains WR and IHDW)-, ectromelia virus (EV)-, cowpox virus (CPV)-, and rabbitpox virus (RPV)-infected cells were chemically cross-linked to ^{32}P-rabbit IFN-γ. Location of monomer and dimer forms of IFN-γ are indicated with arrows, as is the labeled complex between the poxviral IFN-γ binding proteins and radiolabeled rabbit IFN-γ. Molecular weight marker sizes are indicated on the left.

Binding of interferon-γ to the surface of uninfected cells

Binding of radiolabeled human, rabbit, and murine IFN-γ to BGMK cells in monolayer culture was performed essentially as described (Zoon *et al.*, 1986). Briefly, 1×10^4 BGMK cells seeded in 24-well dishes were washed with ice-cold serum-free medium, followed by the addition of increasing amounts of ^{32}P-IFN-γ in 100 μl serum-free medium, either alone or in the presence of 100-fold excess unlabeled IFN-γ. The cultures were incubated at 4° with gentle rocking for 1 hr, at which time the monolayers were washed three times with PBS to remove unbound ^{32}P-IFN-γ. Cells were removed with 0.15 *M* sodium chloride and 0.015 *M* sodium citrate, and the radioactivity in the samples was determined by Cerenkov counting. Specific binding was calculated by subtracting nonspecific binding in the presence of excess unlabeled IFN-γ from total binding. All assays were performed in triplicate.

RESULTS

Many poxviruses express soluble IFN-γR homologs

Due to the accumulation of genomic sequence information, putative IFN-γR homologs have recently been discovered to be encoded by a variety of poxviruses (reviewed in Mossman *et al.*, 1995a). Figure 1 demonstrates the presence of complexes containing radiolabeled rabbit IFN-γ bound to secreted proteins from the supernatants of cells infected with myxoma, SFV, vaccinia (strains WR and IHDW), ectromelia, cowpox, and rabbitpox viruses (Fig. 1, lanes 2–8, respectively), which are not seen in mock-infected supernatants (Fig. 1, lane 1). In other studies, all vaccinia virus strains tested possess a com-

parable IFN-γ binding protein (A. Alcamí and G. Smith, personal communication). In the cross-linking assays shown in Fig. 1, monomer, dimer, and higher molecular weight complexes of IFN-γ are detected. Frequently, the intensity of dimeric IFN-γ decreases upon the formation of IFN-γ/IFN-γR homolog complexes, which might suggest that the IFN-γ binding proteins are preferentially disrupting IFN-γ dimers. Cross-linking assays, while useful for demonstrating protein associations, do not necessarily reflect all of the biological interactions between protein species. Thus, the decrease in intensity of dimeric IFN-γ observed in Fig. 1 does not formally prove that the ligand dimer is the preferential substrate for the formation of IFN-γ/IFN-γR homolog complexes. The issue of monomeric versus dimeric IFN-γ specificity for the poxviral IFN-γR homologs remains to be addressed. The sizes of the shifted complexes are consistent with the predicted molecular mass of the known viral proteins complexed to the IFN-γ ligand, taking into consideration post-translational modifications such as glycosylation, which could account for some of the minor bands observed. The myxoma virus M-T7 protein, the most abundantly secreted protein from myxoma virus-infected cells, demonstrated by the intensity of the labeled complex, is known to be N-glycosylated (K. Mossman and G. McFadden, unpublished). Thus, it appears that the expression of a soluble IFN-γ receptor homolog is a common feature used by poxviruses to counteract the pleiotropic effects of IFN-γ.

Characterization of the ectromelia virus IFN-γR homolog

Since IFN-γ was found to be essential for the clearance of ectromelia virus in a murine model (Karupiah *et al.*, 1993a), we sought to further characterize the ectromelia virus IFN-γ binding protein observed in Fig. 1. DNA sequencing of the ectromelia virus genome revealed the presence of a complete open reading frame related to the myxoma virus M-T7 gene and the vaccinia virus B8R gene (Fig. 2A). The DNA sequence 5′ of the ectromelia virus open reading frame is consistent with transcription at early times postinfection (Davison and Moss, 1989) and an early transcription termination signal (TTTTTAT) is present 30 nucleotides 3′ of the open reading frame. As expected, the ectromelia virus IFN-γ binding protein is most closely related to the homologs from vaccinia and variola viruses (Fig. 2B) since all three are orthopoxviruses, whereas myxoma virus belongs to the leporipoxviridae. While the poxviral IFN-γ binding proteins contain relatively low homology (~20%) to the ligand binding domain of the human and murine IFN-γ receptors, high homology is observed within the orthopox and leporipox genera (Mossman *et al.*, 1995b). The lengths of these proteins are very similar and the number of amino acid differences between the ectromelia–vaccinia, ectro-

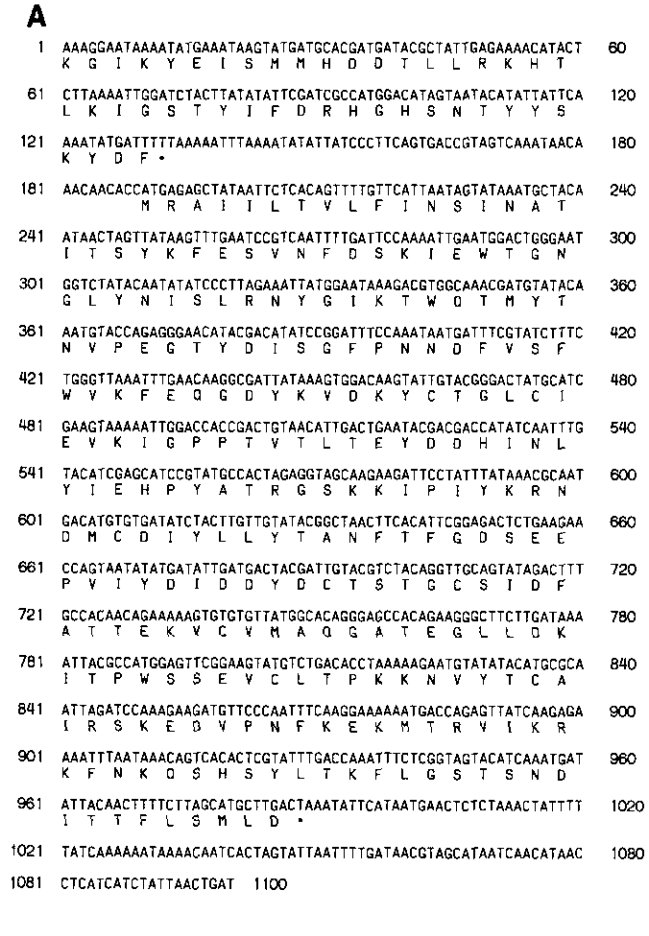

FIG. 2. Ectromelia virus IFN-γ receptor homolog. (A) DNA sequence and translation of a region encompassing the ectromelia virus IFN-γ receptor homolog gene. The partial upstream ORF is homologous to vaccinia virus B7R of vaccinia virus (strain Copenhagen). Asterisks indicate the location of stop codons. (B) Amino acid alignment of IFN-γ receptor homologs encoded by ectromelia virus, vaccinia virus (VV, strain Copenhagen), and variola virus (VAR, strain India 1967). Conserved cysteines believed to be involved in formation of stabilizing disulfide bonds are boxed. Closed circles denote the location and number of amino acid changes between the three peptide sequences. The gap introduced to provide maximal alignment is indicated with a small dot.

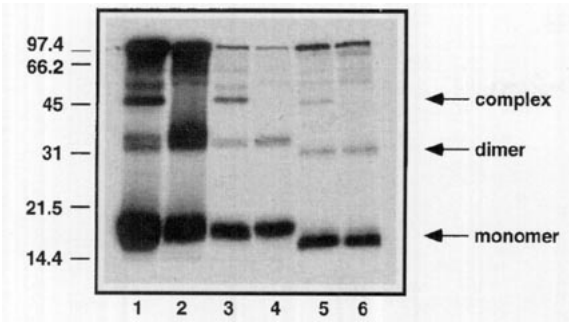

FIG. 3. Ectromelia virus IFN-γ receptor homolog binds multiple IFN-γ species. Supernatants from ectromelia virus-infected cells (lanes 1, 3, and 5) and mock-infected cells (lanes 2, 4, and 6) were chemically cross-linked to ^{32}P-rabbit, -human, and -murine IFN-γ (lanes 1–2, 3–4, 5–6, respectively). Location of monomer and dimer forms of IFN-γ are indicated with arrows, as is the labeled complex formed by the ectromelia virus IFN-γ binding protein and the labeled ligand. Molecular weight marker sizes are indicated on the left.

melia–variola, and vaccinia–variola viral pairs are 19, 23, and 18, respectively. In particular, while the myxoma virus M-T7 protein contains all eight conserved cysteines believed to be important for the structure of the ligand binding domain (Mossman *et al.*, 1995a,b), only six of the eight cysteines are conserved in each of the orthopoxviruses, with the first two cysteines replaced by tyrosines in each case.

Species specificity of ectromelia virus IFN-γ binding protein

Unlike the myxoma virus M-T7 protein, which is specific for binding rabbit IFN-γ (Mossman *et al.*, 1995b), the ectromelia virus IFN-γ binding protein does not exhibit comparable species specificity (illustrated in Fig. 3). The IFN-γ binding protein present in ectromelia virus-infected supernatants is capable of binding rabbit, human, and murine IFN-γ (lanes 1, 3, and 5, respectively), while no binding with these three ligands is seen in mock-infected supernatants (lanes 2, 4, and 6, respectively). The relative intensities of the shifted complexes might suggest that the ectromelia virus IFN-γ binding protein preferentially binds rabbit IFN-γ, but consideration must be given to the number of phosphorylation sites (rabbit and human IFN-γ have two sites, murine IFN-γ has one) and to the differential radiolabeling at these sites, which is reflected by the monomeric IFN-γ levels.

To further characterize the species specificity of the ectromelia virus IFN-γ binding protein, chemical cross-linking assays involving cross-species competition were performed. Supernatants from ectromelia virus-infected cells were incubated with ^{32}P-human, -murine, and -rabbit IFN-γ, either alone or in the presence of increasing amounts of unlabeled competitor IFN-γ. As seen in Fig. 4A, the ectromelia virus IFN-γ binding protein efficiently binds ^{32}P-human IFN-γ (lane 1), consistent with previous results (Fig. 3), and the labeled human IFN-γ is comparably competed from the complex by unlabeled rabbit, human, and murine IFN-γ (lanes 2–13). In all cases, complete competition is observed with a 40-fold excess of unlabeled IFN-γ (lanes 5, 9, and 13). Similar results are seen when ^{32}P-murine IFN-γ (Fig. 4B) or ^{32}P-rabbit IFN-γ (Fig. 4C) are used as the radiolabeled ligand. Thus, it appears that the ectromelia virus IFN-γ binding protein has similar affinities for human, murine, and rabbit IFN-γ.

Species specificity of the vaccinia virus IFN-γ binding protein

Since vaccinia virus also expresses an IFN-γ binding protein (Fig. 1, lanes 4 and 5), it was of interest to determine the specificity of this protein, using the same cross-species competition cross-linking assay described above. Like the ectromelia virus IFN-γ binding protein, the vaccinia virus homolog is capable of binding radiolabeled human, murine, and rabbit IFN-γ (lane 1 in Figs. 5A–5C, respectively). However, the vaccinia virus IFN-γ binding protein does not have the same relative affinity for all three ligand species. As seen in Fig. 5A, excess unlabeled rabbit IFN-γ (lanes 2–5) and human IFN-γ (lanes 6–9) successfully compete away the labeled human ligand from the complex, whereas excess unlabeled murine IFN-γ (lanes 10–13) is unable to function as a competitor, even at a 40-fold excess over the labeled human ligand (lane 13). Similar results are seen if rabbit IFN-γ replaces human IFN-γ as the radiolabeled ligand (Fig. 5C). Accordingly, excess unlabeled rabbit and human IFN-γ successfully compete away ^{32}P-murine IFN-γ from the labeled complex (Fig. 5B). Complete competition is seen with a 10-fold excess of rabbit and human IFN-γ (lanes 3 and 7, respectively), while a 40-fold excess of unlabeled murine IFN-γ is necessary to compete with its labeled counterpart to the vaccinia virus IFN-γ binding protein (lane 13). Thus, the putative vaccinia virus IFN-γR homolog has reduced relative affinity for murine IFN-γ compared to the affinity for either rabbit or human IFN-γ.

Binding of IFN-γ is specific to secreted viral proteins

Soluble forms of mammalian IFN-γ cellular receptors are known to be shed from cells (Novick *et al.*, 1989; Rose-John and Heinrich, 1994), and thus the question arises of whether the detected soluble IFN-γ binding proteins are virus encoded. It is conceivable that soluble forms of the cellular receptor could be shed as a result of viral infection, for example, by increased cell surface proteolysis, and thus the binding observed in the cross-linking assays might not be specific to virally secreted proteins. Since all of the viral supernatants were cultured from monkey kidney cell lines, it was important to ascertain the species-specific binding properties of monkey kidney cellular receptors. As seen in Fig. 6, monolayers

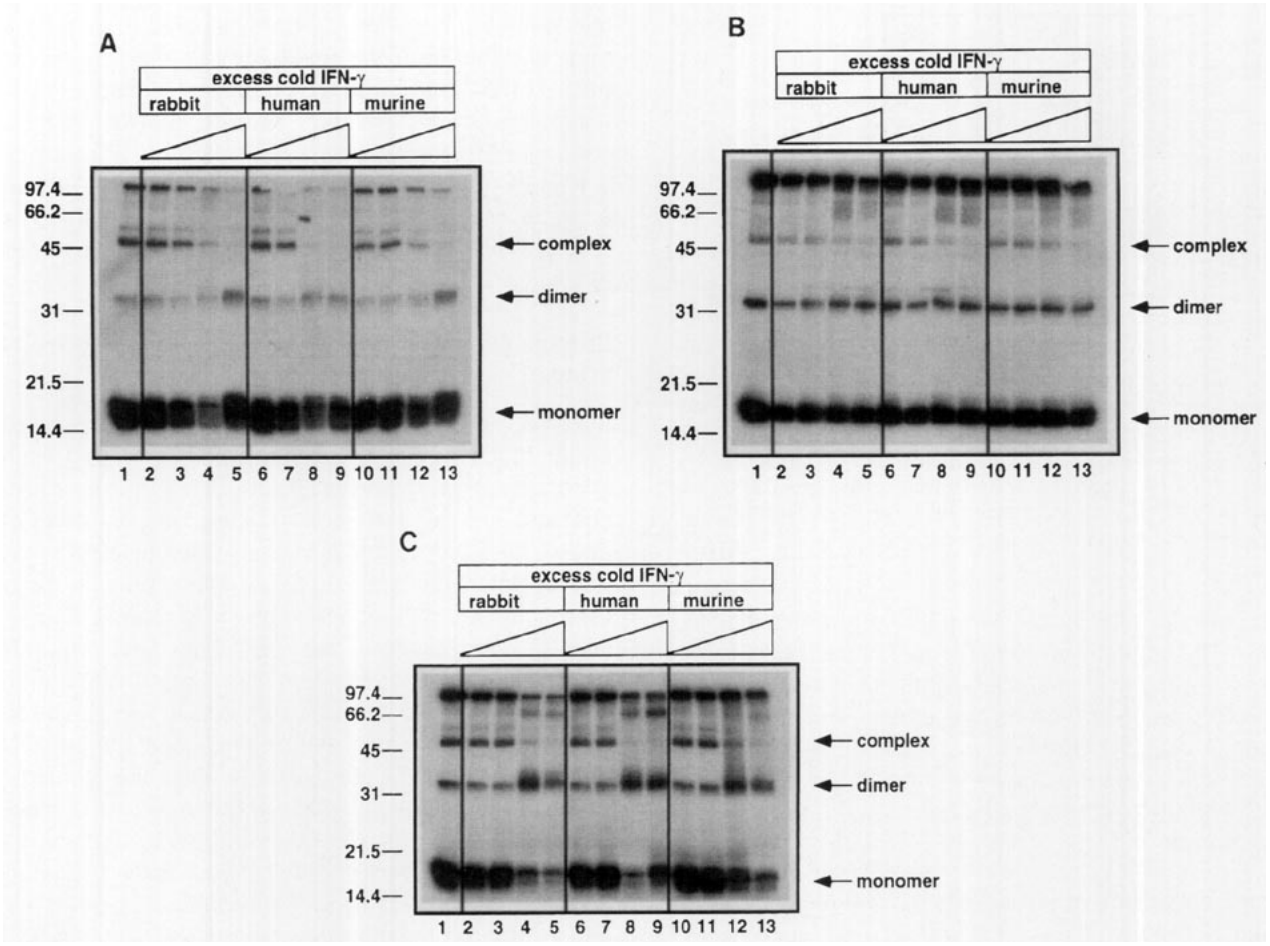

FIG. 4. Species specificity of the ectromelia virus IFN-γ binding protein. Supernatants from ectromelia virus-infected cells were chemically cross-linked to (A) ^{32}P-human IFN-γ, (B) ^{32}P-murine IFN-γ, and (C) ^{32}P-rabbit IFN-γ. In all panels, chemical cross-linking was performed in the absence (lane 1) or presence (lanes 2–13) of increasing amounts of unlabeled IFN-γ, as illustrated. Addition of unlabeled competitor IFN-γ was in 5, 10, 20 and 40× excess over the amount of labeled IFN-γ. Location of monomer and dimer forms of IFN-γ are indicated on the right, while molecular weight marker sizes are indicated on the left.

of BGMK cells are capable of binding human and rabbit IFN-γ, but not murine IFN-γ. Since both ectromelia virus and vaccinia virus are capable of binding murine IFN-γ, albeit with different affinities, shed cellular receptors cannot be responsible for the binding of IFN-γ observed in poxvirus-infected supernatants. Furthermore, the migration of the shifted complexes observed in Fig. 1 vary with the different viruses, which is inconsistent with shed cellular receptors being solely responsible for IFN-γ binding. Although one cannot formally rule out the possibility that viral infection does indeed cause shedding of cellular receptors and that a portion of the binding observed is due to these shed receptors, at least in the case of myxoma virus, disruption of the M-T7 gene results in complete loss of IFN-γ binding proteins in the supernatant of virus-infected cells (Mossman *et al.*, in preparation).

DISCUSSION

For successful propagation and survival, poxviruses have evolved numerous strategies to circumvent the host immune system (for reviews see Buller and Palumbo, 1991; Smith, 1994; Spriggs, 1994; McFadden, 1995). IFN-γ is a critical regulator of the immune system, and the importance of this pleiotropic cytokine in the immune response to poxvirus infection has been clearly demonstrated (Kohonen-Corish *et al.*, 1990; Ruby and Ramshaw, 1991; Buller and Palumbo, 1991; Ramshaw *et al.*, 1992; Karupiah *et al.*, 1993a,b; Melkova and Esteban, 1994). As such, mechanisms to combat the potent anti-viral effects of IFN-γ would certainly be advantageous to poxviruses. While the myxoma virus M-T7 protein is the first *bona fide* IFN-γR homolog shown to actually inhibit IFN-γ, comprehensive sequencing analysis has suggested the presence of putative IFN-γ binding proteins in the genomes of SFV, vaccinia virus, variola virus, and swinepox virus (Upton *et al.*, 1992; Goebel *et al.*, 1990; Shchelkunov *et al.*, 1993; Massung *et al.*, 1993, 1994).

In this report, we demonstrate that myxoma virus is not unique in elaborating an IFN-γR homolog and that the ability to combat the immunomodulatory effects of IFN-γ by secreting an IFN-γ binding protein appears to

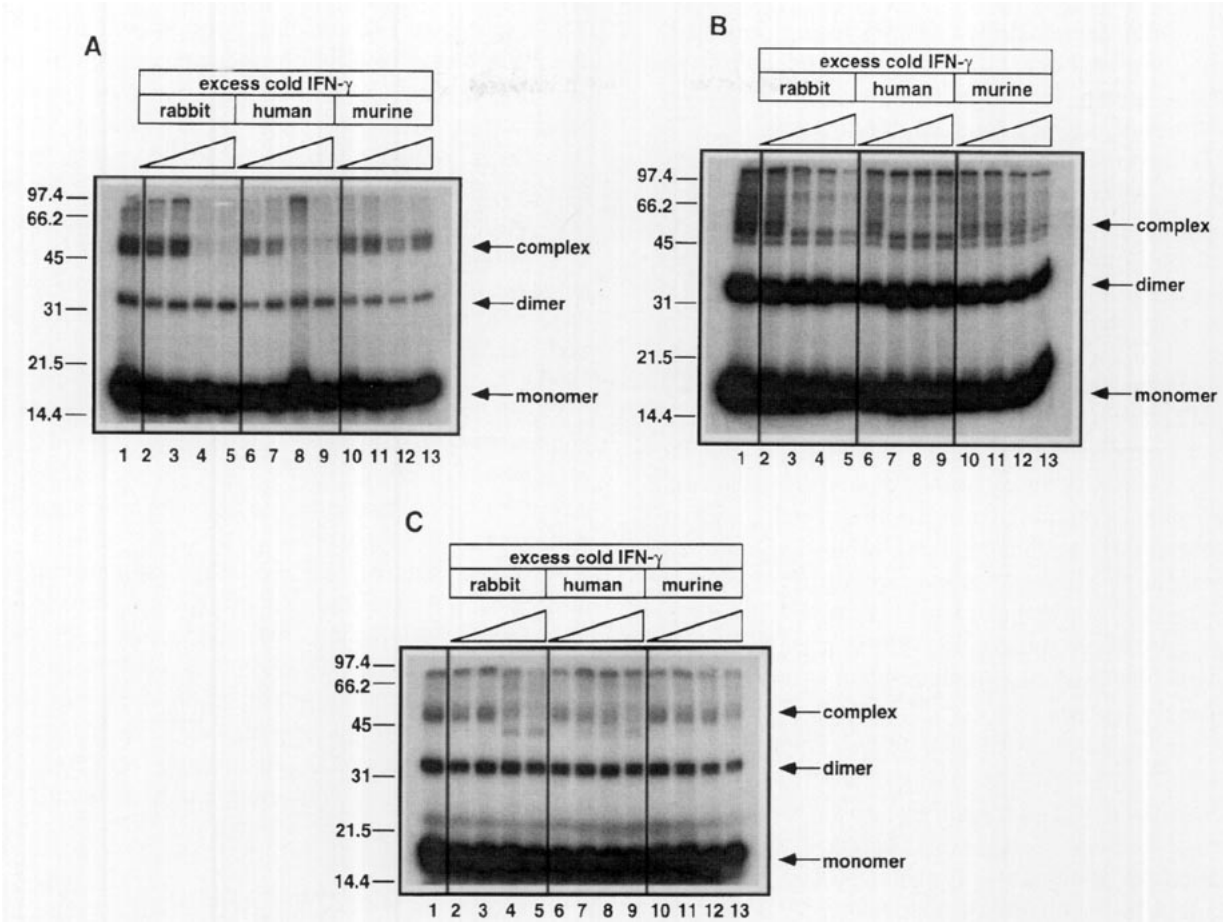

FIG. 5. Species specificity of the vaccinia virus IFN-γ binding protein. Supernatants from vaccinia virus-infected cells were chemically cross-linked to (A) ^{32}P-human IFN-γ, (B) ^{32}P-murine IFN-γ, and (C) ^{32}P-rabbit IFN-γ. In all panels, chemical cross-linking was performed in the absence (lane 1) or presence (lanes 2–13) of increasing amounts of unlabeled IFN-γ, as illustrated. Addition of unlabeled competitor IFN-γ was in 5, 10, 20, and 40× excess over the amount of labeled IFN-γ. Location of monomer and dimer forms of IFN-γ are indicated on the right, while molecular weight marker sizes are indicated on the left.

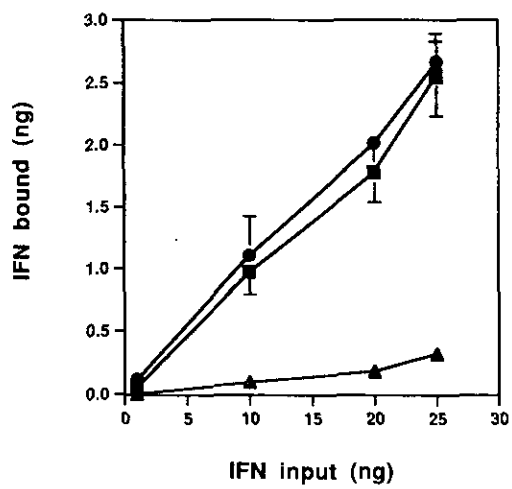

FIG. 6. IFN-γ binding specificity of primate BGMK cells. Binding analysis of ^{32}P-human IFN-γ (■), ^{32}P-rabbit IFN-γ (●), and ^{32}P-murine IFN-γ (▲) to the primate IFN-γ receptor on intact BGMK cells. Specific binding reflects total binding of radiolabeled ligand minus nonspecific binding in the presence of 100-fold excess unlabeled ligand. All binding assays were done in triplicate.

be a characteristic conserved among many, if not all, poxviruses. Cross-linking assays using radiolabeled rabbit IFN-γ and supernatants from a variety of poxviral-infected cells illustrated that all of the poxviruses tested express an IFN-γ binding protein, similar in molecular mass to the 37-kDa myxoma virus M-T7 protein. Since the clearance of ectromelia virus within a murine model was found to be dependent on the presence of IFN-γ (Karupiah *et al.*, 1993a), expression of an IFN-γ binding protein by ectromelia virus and discovery of the corresponding open reading frame was not surprising. Although studies of mammalian soluble IFN-γRs have characterized physiological complexes to consist of two soluble receptors to one IFN-γ homodimer (Greenlund *et al.*, 1993), it must be emphasized that while cross-linking competition assays are useful for indicating the hierarchy of binding affinities, they are unable to quantitate the stoichiometry of interaction. Further experiments are currently in progress to address this question.

The issue of ligand specificity has relevance to virus evolutionary history. Myxoma virus, which has evolved in the tapeti, or South American brush rabbit (Fenner and

Ratcliffe, 1965), demonstrates strict species specificity for binding and inhibiting rabbit IFN-γ and possesses no measurable affinity for either human or murine IFN-γ (Mossman *et al.*, 1995b). As such, the myxoma virus M-T7 protein is similar to the mammalian cellular receptor complexes, which demonstrate specificity in binding their cognate ligands (Aguet *et al.*, 1988; Gray *et al.*, 1989). Since myxoma virus is one of the few poxviruses for which an evolutionary origin is firmly established, it is feasible to speculate that interactions involving poxviral binding proteins, such as the IFN-γR homologs, may lend insight into the evolutionary origin of other poxviruses. In light of both the species-specific nature of the myxoma virus M-T7 protein for the rabbit IFN-γ ligand and the necessity of IFN-γ in the clearance of an ectromelia virus infection within a murine model, it was surprising to demonstrate that the ectromelia virus IFN-γR homolog possesses the same relative affinity for all three ligands tested. The question is thus raised concerning the evolutionary origin of ectromelia virus. Potentially, either ectromelia virus has been passaged within a series of vertebrate hosts, thus obtaining comparable affinity for a variety of ligand species, or its evolutionary host is equally diverged from rabbits, mice, and humans.

Vaccinia virus, widely used as a vaccine against variola virus, the causative agent of smallpox, is a prominent poxvirus whose natural history remains obscure. Thus it is important to ascertain the specificity of the vaccinia virus IFN-γR homolog in an attempt to clarify the evolutionary history of this virus. Due to the high degree of homology between the three known orthopoxvirus IFN-γ binding proteins, it was of interest to discover a significant difference in the relative affinity of binding to murine IFN-γ between the vaccinia virus and ectromelia virus IFN-γR homologs. In independent studies, it has been shown that the vaccinia virus IFN-γ binding protein from strains other than the WR and IHDW strains tested here do not significantly interact with murine IFN-γ (A. Alcamí and G. Smith, personal communication). Thus, it appears that the evolution of vaccinia virus is not as obvious as that of myxoma virus and that identification of likely vertebrate hosts of the progenitor of vaccinia virus must await a survey of other mammalian IFN-γ ligands and analysis of the relevant dissociation constants, using purified viral receptor homologs.

The comparative study of members of the poxvirus family is an important endeavor as it contributes to our understanding of the evolution of the molecular basis of virus virulence. Over millions of years of evolution, these viruses have flourished by establishing stable relationships with their hosts, in part by elaborating a plethora of anti-immune strategies in order to minimize immune recognition and clearance. One such strategy is the production of soluble IFN-γR homologs (Upton *et al.*, 1992), which counteract the potent and pleiotropic effects of IFN-γ, a cytokine which has been shown to be most important in the recovery of the mouse from infections with the natural viral pathogens, such as murine cytomegalovirus (Lucin *et al.*, 1992) and ectromelia virus (Karupiah *et al.*, 1993a). Although the systemic recovery from ectromelia virus infection was dependent in part on the ability of IFN-γ to induce the enzyme nitric oxide synthase, responsible for generation of nitric oxide, a potent anti-viral molecule (Karupiah *et al.*, 1993b), IFN-γ has apparently little anti-viral effect at the primary infection and transmission site in the foot-pad (Karupiah *et al.*, 1993a; Lucin *et al.*, 1992). Conceivably, in the first 24 hr of the infection, the rate of production of ectromelia virus soluble IFN-γR homolog in this peripheral site of the skin is sufficient to neutralize the endogenously produced IFN-γ, thereby attenuating and delaying the acute inflammatory response.

Poxviruses constitute a potentially valuable repository of both cytokine homologs and cytokine inhibitor homologs (Smith, 1994; Spriggs, 1994; Mossman *et al.*, 1995a). However, in order to fully comprehend the role(s) these inhibitors play, a further appreciation of the evolutionary origins of these viruses becomes exceedingly important. Fortunately, the poxvirus system is an attractive one in which to study such virus–host interactions in order to address this issue.

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