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SOLUBLE INTERFERON-γ RECEPTORS ENCODED BY POXVIRUSES

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Abstract—Poxviruses encode a broad range of proteins that counteract the formidable attack of the immune response initiated in the host after infection, among which are proteins that mimic the extracellular binding domain of host cytokine receptors and are secreted from virus-infected cells. A soluble interferon-γ receptor (IFN-γR) is produced early after infection and efficiently blocks the binding of IFN-γ to cellular receptors, thus inhibiting both the anti-viral and immune functions of IFN-γ. An IFN-γR is highly conserved among members of the poxvirus family, suggesting a major role in viral pathogenesis. The highly species-specific nature of the IFN system enables questions concerning the evolutionary relationship between poxviruses and their hosts to be addressed. The IFN-γR encoded by myxoma virus, a natural pathogen of rabbits, is specific for rabbit IFN-γ. However, the IFN-γR encoded by orthopoxviruses (vaccinia, cowpox, camelpox, ectromelia) shows a novel, broad species specificity suggesting that these viruses have evolved in several species. The implications for the unknown origin and natural host(s) of vaccinia virus are discussed. Copyright © 1996 Elsevier Science Ltd

Key words: Cytokine receptor, immune evasion, interferon, pathogenesis, poxvirus, vaccinia, virus evolution.

Résumé—Les Poxvirus suscitent l'apparition d'une grande quantité de protéines qui neutralise la formidable attaque de la résponse immune initiée chez l'hôte après l'infection. Parmi ces proteines, certaines imitent la partie extérieure des cytokines de l'hôtes et sont élaborées par le virus. Un récepteur soluble de l'interferon γ (IFN-γR) esi produit très tôt apres l'infection et bloque la liason récepteur cellulaire—IFN γ). Un IFR-γR est très présent parmi les membres de la famille des Pox, suggérant un rôle majeur dans la pathogènie virale. La spécificité du système de l'IFN autorise à se poser des questions concernant l'évolution des relations Pox-virus-hôtes. Le IFN-γR produit par le virus du myxome, virus pathogène pour le lapin, est spécifique de l'IFN γ. Cependant, l'IFN-γR produit pare les erthopoxvirus (vaccine, cowpos, camelpox, etromelie) montrent une spécificité d'espèce très large suggérnant que ces virus se sont développés dans plusieurs espèces. Les implications relativés à l'origine inconnue et aux hôtes naturels du virus de la vaccine sont discutées.

Mots-clefs: Soluble interferons-γ.

POXVIRUSES

Poxviruses are complex DNA viruses that replicate in the cytoplasm and normally produce an acute infection in the host [1, 2]. Poxviruses have played important roles in the history of medicine. Variola virus, which caused smallpox, was a strictly human pathogen and until its eradication produced one of the most devastating of human diseases. Cowpox

Abbreviations used: 2′5′-A, 2′5′-oligoadenylate; dsRNA, double-stranded RNA; IFN, interferon; IFN-α/βR, IFN-α/β receptor; IFN-γR, IFN-γreceptor; IL-1β, interleukin-1β; IL-1βR, IL-1β receptor; kb, kilobase; kDa, kilodalton; ORF, open reading frame; PKR, dsRNA-dependent protein kinase; TNF, tumour necrosis factor; TNFR, TNF receptor; WR, Western Reserve.

virus was introduced by Jenner in 1798 as a smallpox vaccine but, in the 20th century, vaccinia virus was the vaccine used world-wide by the World Health Organization to achieve the global eradication of smallpox by 1977 [3]. Members of the *Poxviridae* family are classified in genera (Table 1), such as *Orthopoxvirus* (cowpox, vaccinia, variola, ectromelia and camelpox viruses), *Leporipoxvirus* (myxoma and Shope fibroma viruses), *Suipoxvirus* (swinepox virus) and *Capripoxvirus* (sheeppox virus).

Cowpox virus is an *Orthopoxvirus* that was so named because it was first isolated from lesions on infected cattle. However, the virus is not presently enzootic in cattle and it is believed that cowpox virus infections of cattle were uncommon during the 18th and 19th centuries [4, 5]. The virus has caused sporadic infections in cows, humans, a wide range of zoo animals and domestic cats, but such outbreaks may not have importance for perpetuation of the virus in nature. It is thought that the natural reservoir of cowpox virus is probably wild rodents from which the virus has been isolated [4, 5]. The origin of vaccinia virus remains unclear and its natural host is unknown [1, 3–5]. Although Jenner introduced cowpox for vaccination against smallpox in 1798, the smallpox vaccines used this century were derived from a different species, not presently found in nature, and were named vacinia after the vaccination procedure [6]. Following the extensive use of vaccinia for smallpox vaccination, the virus has infected domestic animals, notably buffalos in India. Buffalopox, regarded as a subspecies of vaccinia virus, has been maintained in the buffalo population and transmitted to humans on occasions. Despite this, vaccinia virus is not considered a natural human pathogen and is transmitted poorly between humans. Previous suggestions that the virus might have derived from either cowpox or variola virus are now considered unlikely following analyses of the biological properties, restriction enzyme maps and DNA sequences of these viruses, which have demonstrated that vaccinia virus is a distinct species of *Orthopoxvirus* [5, 7–15]. Ectromelia virus is an *Orthopoxvirus* that has been isolated from laboratory mouse colonies on several occasions. The virus shows a narrow host range and its natural reservoir is probably wild mice. Camelpox virus causes a natural systemic infection in camels.

Myxoma virus, a *Leporipoxvirus*, has probably co-evolved with wild American rabbits and establishes a benign infection in these hosts that enables the virus to persist in localized dermal lesions [16]. In contrast, myxoma virus is highly virulent in the European rabbit, producing a systemic lethal infection named myxomatosis. Shope fibroma virus produces a localized benign fibroma in rabbits. Swinepox is the only known member of the *Suipoxvirus* genus and produces a mild infection in pigs. The *Capripoxvirus* genus includes sheeppox virus [2].

Table 1. Genera and species of poxvirus

Genus	Species	Host range	Natural host
Orthopoxvirus	Vaccinia	Wide	Unknown, isolated from animals (rabbitpox, buffalopox)
	Cowpox	Wide	Sporadic infections in cows, rodents, cats, zoo animals and humans
	Variola	Narrow	Humans
	Ectromelia	Narrow	Mice
	Camelpox	Narrow	Camels
Leporipoxvirus	Myxoma	Narrow	Rabbits
	Shope fibroma	Narrow	Rabbits
Suipoxvirus	Swinepox	Narrow	Pigs
Capripoxvirus	Sheeppox	Narrow	Sheep, goats

POXVIRUS MECHANISMS FOR IMMUNE EVASION

The genome of poxviruses is a large double-stranded DNA molecule that varies from 130 to 300 kilobases (kb) [2]. The complete genome of vaccinia virus strain Copenhagen and variola virus strains Bangladesh-1975 and India-1967 have been sequenced and shown to encode approximately 200 proteins [8, 10–15]. Many of these proteins are essential for virus growth in tissue culture, whereas others, mostly encoded by genes located near the ends of the virus genome, facilitate virus replication *in vivo* or interfere with host immune functions [17–23].

Cytokines are soluble mediators of the immune system that play important roles in anti-viral defences and thus it is not surprising to find that poxviruses have evolved a variety of mechanisms that interfere with the activity of host cytokines (Table 2). These may function intracellularly or extracellularly. The cowpox protein crmA (cytokine response modifier A) and vaccinia protein B13R are intracellular proteins that inhibit the interleukin-1β (IL-1β) converting enzyme, which cleaves pro-IL-1β to generate mature active IL-1β [24] (S. Kettle and G. L. Smith, unpublished). The intracellular vaccinia virus proteins E3L [25–28] and K3L [28–30], which are conserved in variola virus [13, 31], block interferon (IFN)-mediated responses and protect the infected cell from the anti-viral effects of IFNs.

Another anti-cytokine mechanism used by poxviruses is the secretion of soluble versions of cytokine receptors that intercept the normal activities of the target cytokines [18–23]. The term "viroceptor" has been proposed for these virus-encoded homologues of cellular cytokine receptors [32]. A soluble tumour necrosis factor receptor (TNFR) is secreted from cells infected by myxoma, Shope fibroma and cowpox viruses [32–34], and a TNFR gene predicted to encode an active TNFR has been found in variola virus [9, 13, 31, 35]. TNFR genes are mutated in vaccinia virus strains Western Reserve (WR) and Copenhagen [8, 36], but a TNF binding activity is encoded by the Lister strain of vaccinia virus (A. Alcamí and G. L. Smith, unpublished). Vaccinia and cowpox viruses express a soluble receptor for IL-1β (IL-1βR) [37, 38]. The activity of IFNs are also blocked by soluble receptors. Myxoma virus encodes an IFN-γ receptor (IFN-γR; [39]) that is expressed by several orthopoxviruses [40, 41], and an IFN-α/β receptor (IFN-α/βR) has been described in vaccinia, cowpox, camelpox and ectromelia viruses [42, 43]. Genes predicted to encode IFN-γRs and IFN-α/βRs have been sequenced in variola virus [13, 31]. An open reading frame (ORF) predicted to encode a chemokine receptor-like molecule has been described in swinepox virus [44] and sheeppox virus [45], but to date no functional data have been reported.

IFN-γ AND ITS RECEPTOR

IFN-γ, a pleiotropic cytokine with anti-viral and immunoregulatory effects [46, 47], is a secreted polypeptide with a predicted molecular mass of 17 kDa that is glycosylated and exhibits monomer sizes of 20 and 25 kDa in purified natural preparations. Biologically active IFN-γ is a noncovalent homodimer and its three-dimensional structure has been reported [48] (Fig. 1).

IFN-γ exerts its activity by interacting with specific receptors on the target cell. The mature human IFN-γR is a 472 amino acid membrane glycoprotein of 80–95 kDa that belongs to the type II cytokine receptor family [49, 50] (Fig. 1). The extracellular domain consists of 228 amino acids, including ten cysteine residues and five potential N-linked

Table 2. Anti-cytokine strategies encoded by poxvirus*

Cytokine	Mechanism†	Virus factor‡	Virus	Reference
IL-1β	Inhibits the IL-1β converting enzyme	crmA (cow), B13R (vac)	Cowpox, vaccinia	20; S. Kettle and G. L. Smith, unpublished
IFN-$\alpha/\beta/\gamma$	Blocks PKR activation by dsRNA	E3L (vac), E3L (var)	Vaccinia, variola	10, 11, 20–24
IFN-$\alpha/\beta/\gamma$	Prevents phosphorylation of eIF2α by PKR	K3L (vac), C3L (var)	Vaccinia, variola	10, 11, 24–26
TNF	Soluble receptor	T2 (myx), crmB (cow), G2R (var)	Myxoma, Shope fibroma, cowpox, vaccinia, variola	7, 8, 10, 11, 27–31; A. Alcamí and G. L. Smith, unpublished
IL-1β	Soluble receptor	B15R (vac)	Vaccinia, cowpox	32, 33
IFN-γ	Soluble receptor	T7 (myx), B8R (vac), C6L (swi), B8R (var)	Myxoma, vaccinia, cowpox, camelpox, ectromelia, swinepox, variola	34–36
IFN-α/β	Soluble receptor	B18R (vac), B17R (var)	Vaccinia, cowpox, camelpox, ectromelia, variola	37, 38
Chemokines	Membrane chemokine receptor-like protein	K2R (swi), Q2/3L (she)	Swinepox, sheeppox	39, 40

**Abbreviations*: dsRNA, double stranded RNA; eIF2α, eukaryotic initiation factor 2α; IFN, interferon; IL-1β, interleukin-1β; PKR, dsRNA-dependent protein kinase; TNF, tumour necrosis factor.

†The activity has been demonstrated in all cases except for the chemokine receptor-like protein, which is predicted from sequence similarity.

‡The virus is indicated in parentheses: cow, cowpox; myx, myxoma; she, sheeppox; swi, swinepox; vac, vaccinia strain Copenhagen (except for B15R and B18R which is strain WR); var, variola strain Bangladesh-1975.

glycosylation sites, and is sufficient to confer IFN-γ binding with high affinity. The intracellular domain consists of 220 amino acids and is devoid of intrinsic kinase or phosphatase activity. The mouse IFN-γR shows 52.5% amino acid identity with its human counterpart, and both are organized in a similar manner [47]. Cell signalling requires the IFN-γ-receptor complex to interact with at least one additional species-specific receptor component, which has been cloned and shown to belong to the type II cytokine receptor family [51]. It is believed that interaction of the IFN-γ homodimer with two IFN-γRs on the target cell initiates dimerization and provides a binding site for accessory factors involved in signal transduction [46, 47] (Fig. 1).

Soluble versions of the extracellular binding domain of the IFN-γR have been produced and shown to bind IFN-γ with high affinity and to prevent the interaction of IFN-γ with receptors on the cell surface [52, 53]. The three-dimensional structure of IFN-γ complexed to the extracellular domain of the receptor has been reported [54]. Soluble IFN-γRs have been found in human urine and may function as physiological regulators of IFN-γ activity *in vivo* [55].

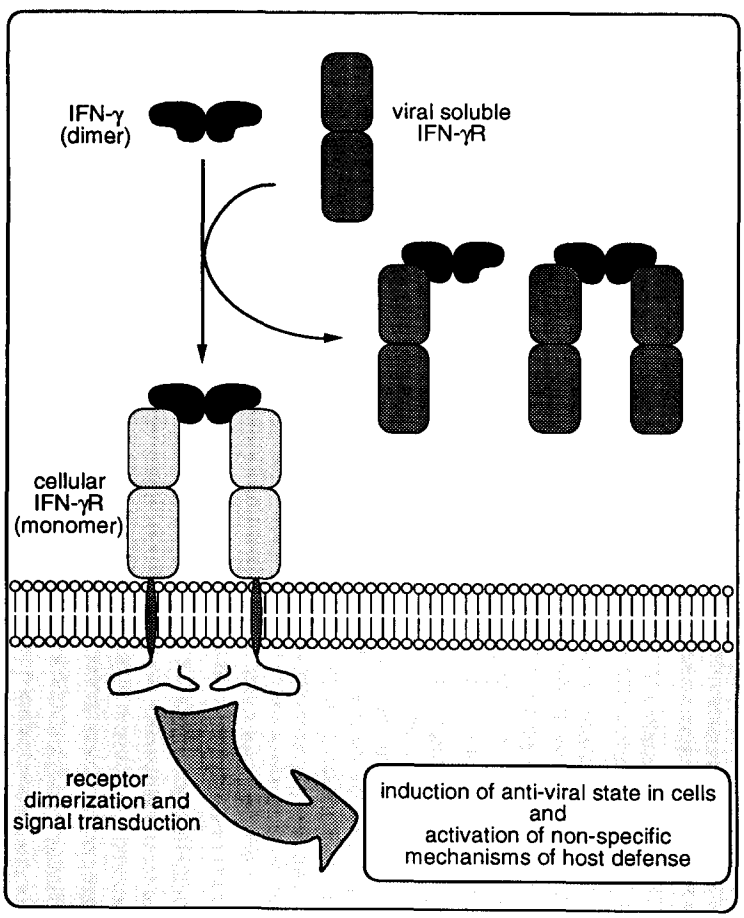

Fig. 1. Representation of the structure and function of IFN-γ and the cellular and viral receptors. See text for details.

IFNs are one of the first lines of host defence [46, 47]. IFN-α/β and IFN-γ induce an anti-viral state in the cell by upregulating the synthesis of several host proteins, which restricts virus replication on subsequent infection. Two IFN-induced enzymes that are activated by double-stranded RNA (dsRNA) have been well characterized (i) the dsRNA-dependent protein kinase (PKR) which phosphorylates the eukaryotic initiation factor 2α, resulting in the inhibition of protein synthesis, and (ii) the 2′5′-oligoadenylate (2′5′-A) synthetase that catalyses the formation of 2′5′-A, which activates ribonuclease L and degrades RNA. In IFN-treated cells, accumulation of dsRNA as a consequence of virus replication activates PKR and 2′5′-A synthetase resulting in the inhibition of protein synthesis and the degradation of viral RNA [56]. In addition to establishing anti-viral pathways, IFN-γ is a potent immunomodulatory cytokine that exerts pleiotropic effects on the immune system, such as promoting cellular immune responses, inducing the synthesis of pro-inflammatory cytokines, increasing antigen presentation and activating the synthesis of nitric oxide by macrophages [46, 47]. While IFN-α/β plays a crucial role in protecting cells from virus infections, the contribution of IFN-γ to anti-viral defence comes mainly from its immunoregulatory activity. For example, IFN-γ upregulates expression of major histocompatibility complex class I molecules and activates cytotoxic T lymphocytes, which in turn destroy infected cells after recognition of viral peptides presented by major histocompatibility complex class I molecules.

With a few exceptions, IFN-γ interacts exclusively with receptors of the same species [46]. Human IFN-γ does not bind to mouse, rat or bovine cells, and mouse IFN-γ does not bind to human cells [46]. Bovine IFN-γ does not have any biological effects on human, rat or mouse cells, only bovine cells [57]. Rat IFN-γ has no activity on human cells, but its high similarity to mouse IFN-γ (87% amino acid identity) probably explains its activity on mouse cells. However, conversely, mouse IFN-γ is not active in rat cells [58, 59].

POXVIRUS SOLUBLE IFN-γ RECEPTORS

The first poxvirus soluble IFN-γR was described in the *Leporipoxvirus* myxoma virus. Upton *et al.* [39] identified the major 37 kDa protein secreted from myxoma-infected cells as the product of the ORF M-T7 and found sequence similarity to the extracellular domain of the human and mouse IFN-γRs (25% amino acid identity). The M-T7 protein lacked the cytoplasmic and membrane-anchor domains present in the cellular IFN-γRs and thus was predicted to be secreted from virus-infected cells (Fig. 1). The M-T7 protein binds rabbit IFN-γ with high affinity (Kd 1.2 nM) and inhibits the anti-viral effects of this cytokine. The myxoma IFN-γR is transcribed early during infection, before viral DNA replication takes place, and is efficiently secreted into the medium (5×10^7 molecules per cell) [41]. The *Leporipoxvirus* Shope fibroma virus ORF S-T7 also encodes an active IFN-γR [41, 60].

Nucleotide sequence of the genome of vaccinia virus strains WR and Copenhagen showed an ORF, named B8R, with sequence similarity (21% amino acid identity) to the myxoma IFN-γR (M-T7) and the extracellular domain of the mouse and human IFN-γRs [8, 36, 39]. The vaccinia B8R ORF has been shown to encode a soluble IFN-γR activity by expression in recombinant baculovirus [40]. Genes related to B8R have been sequenced in ectromelia virus [41], two strains of variola virus [13, 31] and swinepox virus [44]. Cross-linking assays of radiolabelled IFN-γ to supernatants from virus-infected cultures have demonstrated the production of soluble IFN-γRs by 14 strains of vaccinia virus

(including rabbitpox and buffalopox), cowpox (strains Brighton Red and elephantpox), ectromelia and camelpox viruses [40, 41] (Fig. 2). The vaccinia IFN-γR has an apparent size of 43 kDa, deduced from the size of the IFN-γ-receptor complex (60 kDa) by subtraction of the monomer of IFN-γ. This size is larger than that of the myxoma receptor 37 kDa [40] and might reflect the presence of an additional N-glycosylation site in the vaccinia polypeptide compared to that of myxoma [8, 36, 39]. The cowpox, camelpox and ectromelia IFN-γRs show an apparent size similar to the myxoma IFN-γR [40, 41].

The IFN-γR is highly conserved among members of the poxvirus family since each virus tested to date has been shown either to produce a soluble IFN-γ binding protein or to have an IFN-γR gene predicted to encode an active protein.

The presence of a soluble protein that inhibits the anti-viral activity of IFN-γ has been demonstrated in supernatants of cultures infected with myxoma, vaccinia, cowpox and camelpox viruses [39, 40]. This inhibition may be achieved by several possible mechanisms: (i) binding of the virus receptor to the IFN-γ homodimer in solution might block interaction of IFN-γ to the cellular receptor; (ii) the virus IFN-γR might bind to the extracellular domain of the host cell IFN-γR accessory factor and thereby block recruitment of Jak-1 kinase and hence signal transduction [47]; (iii) the IFN-γ-soluble receptor complex might bind to the cell IFN-γR through a second binding site on the IFN-γ homodimer and prevent cell receptor dimerization which is needed to induce signal transduction. However, both (ii) and (iii) are unlikely since virus IFN-γRs are not detected at the cell surface [40]. The blockade of IFN-γ binding to the cell has been formally proven for the vaccinia, cowpox and camelpox virus IFN-γRs [40] (Fig. 1).

ROLE OF THE POXVIRUS SOLUBLE IFN-γR IN VIRUS PATHOGENESIS

The importance of IFN-γ in combating poxviral infections has been illustrated by the demonstration that: (i) the replication of vaccinia and ectromelia viruses in mice is restricted by IFN-γ [61–63]; (ii) mice lacking the IFN-γR are more susceptible to vaccinia virus infections [64, 65]; and (iii) IFN-γ exerts an anti-vaccinia and ectromelia virus activity in macrophages by induction of nitric oxide synthase [66–68]. Thus, the production of a potent IFN-γ inhibitor is predicted to have a profound effect in virus pathogenesis, helping poxviruses to evade IFN-γ-mediated host defence mechanisms.

The contribution of the virus soluble IFN-γR to poxvirus virulence is not yet known since a virus with a disrupted or deleted copy of this gene has not been produced and tested in animal models, but the high conservation of the IFN-γR activity or the encoding gene among poxviruses suggests that the virus IFN-γR plays a major role. Like the vaccinia IL-1βR and IFN-α/βR, and the myxoma TNFR [32, 37, 38, 43], the IFN-γR is predicted to be dispensable for virus replication in tissue culture and, consequently, after continuous passage in the laboratory and without the selective pressure imposed by an animal host, the IFN-γR might have accumulated mutations leading to inactivation in some vaccinia virus strains. However, such assumption is only valid for those virus strains, such as WR, that have been passed extensively in the laboratory. Notably, the smallpox vaccines were grown in the skin of animals and not in cultured cells, even during the Intensified Smallpox Eradication Campaign in the 1970s [3, 5]. These growth conditions may have provided selective pressure for retention of an IFN-γR, suggesting that this cytokine receptor might be required for virus replication in the skin.

The soluble IFN-γR is the fourth vaccinia virus protein shown to counteract the anti-viral effects of IFNs. Vaccinia virus ORFs K3L and E3L encode intracellular proteins that block the IFN-induced inhibition of protein synthesis (Table 2). In contrast, the B8R protein is secreted, binds soluble IFN-γ and prevents its binding to cellular receptors. This strategy to blockade IFN-γ enables poxviruses to inhibit both the anti-viral effects and the more important immune functions of IFN-γ simultaneously. The vaccinia B18R ORF encodes another anti-IFN protein, in this case a soluble IFN-α/βR that blocks the binding of IFN-α/β to cell surface receptors [42, 43].

SPECIES SPECIFICITY OF POXVIRUS IFN-γRs: IMPLICATIONS FOR THE ORIGIN AND NATURAL HOSTS OF POXVIRUSES

In contrast to other cytokines such as IL-1 and TNF, IFNs interact with their receptors in a species-specific manner [46]. Thus the poxvirus IFN-γRs will have ligand specificity that reflects the host(s) in which the virus has evolved. Consistent with this idea, the IFN-γR encoded by myxoma virus, a natural rabbit pathogen, binds rabbit, but not human or mouse, IFN-γ [69].

Interestingly, the vaccinia and cowpox virus IFN-γRs bind human, bovine, rat and rabbit, but not mouse, IFN-γ [40, 41]. This broad species specificity has no precedent among previously described IFN-γRs, which are highly species specific [46]. The binding to rat but not mouse IFN-γ is surprising given that both have a high degree of similarity and suggests that the IFN-γ specificity has been positively selected during virus evolution in different hosts. The binding properties of the vaccinia and cowpox IFN-γRs correlate with the capacity of these viruses to infect a broad range of species both in nature and in the laboratory. Consistent with this, the IFN-α/βR encoded by vaccinia virus has also a broad species specificity, with a lower affinity for mouse IFNα/β [43]. However, ectromelia virus, a strictly mouse pathogen, encodes an IFN-γR with broad species specificity, binding mouse, human and rabbit IFN-γ [41]. Along the same line, variola virus, a strictly human pathogen, encodes an IFN-γR homologue with 91% amino acid identity to the vaccinia B8R ORF [13, 31], suggesting a broad species specificity similar to vaccinia virus. Furthermore, camelpox virus infections occur only in camels yet binding studies indicate a broad IFN-γ species specificity of the camelpox receptor [40]. Although variola, ectromelia and camelpox viruses show a narrow host range in natural infections, limited virus replication has been reported in experimental animals [5], suggesting that other species may have been former hosts for these viruses during evolution. Perhaps an orthopoxvirus ancestor had an IFN-γR with broad species specificity, a property that has been conserved in many members of the genus, although some of these have later evolved a more restricted host range.

The novel broad species specificity of the vaccinia and cowpox IFN-γRs has implications for the origin and natural host(s) of these viruses. Cowpox virus encodes a receptor that binds bovine IFN-γ as expected. The failure of the cowpox receptor to bind mouse IFN-γ suggests that the mouse is not a natural host, but the binding of rat IFN-γ supports the hypothesis that other rodents, such as susliks and gerbils from which cowpox virus has been isolated [70], may constitute a natural reservoir. The ability of both the cowpox and vaccinia receptors to bind the human cytokine suggests that, although these viruses are not considered to be human pathogens, they may have naturally infected humans in the past. The similar binding properties of the vaccinia and cowpox receptors suggest that both

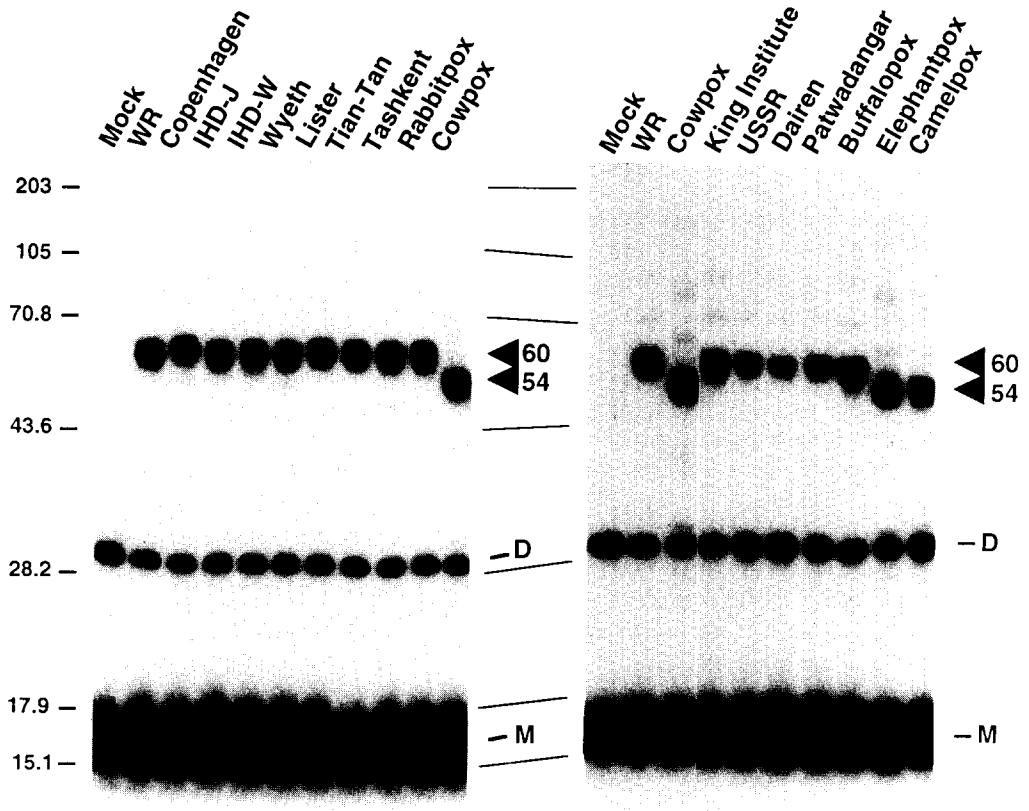

Fig. 2. Expression of soluble IFN-γRs by orthopoxviruses. Cross-linking of ^{125}I-human IFN-γ to medium from cultures infected with vaccinia virus laboratory strains (WR, IHD-J, IHD-W), smallpox vaccines (Copenhagen, Wyeth, Lister, Tian-Tan, Tashkent, King Institute, USSR, Dairen, Patwadangar), vaccinia virus strains isolated from animals (rabbitpox, buffalopox), cowpox virus strain Brighton Red, cowpox virus isolated from an elephant (elephantpox) or camelpox virus. An autoradiograph of the electrophoretic analysis (with molecular masses in kDa) is shown. The positions of the IFN-γ monomer (M), IFN-γ dimer (D) and ligand receptor complexes (arrowhead) are indicated. The cross-linking agent used in this experiment does not introduce covalent bonds between the two molecules of the homodimeric IFN-γ and thus only cross-linked heterodimers of IFN-γ and virus receptor are detected. Reproduced from Ref. [40] with permission.

viruses may have evolved in the same hosts, in agreement with the finding that all the smallpox vaccine strains of the 20th century, isolated independently and thought to be cowpox, are in fact vaccinia virus [6]. This idea supports the favoured hypothesis for the origin of vaccinia virus, which is that vaccinia virus is an independent orthopoxvirus that previously infected species in which it is no longer endemic. Horsepox virus has been suggested, as earlier vaccinators were reported to obtain supplies of vaccine from poxvirus infections of horses when supplies from infected cows were scarce [4].

The reason for vaccinia, and not cowpox, being the orthopoxvirus species present in all the smallpox vaccines used this century probably reflects a higher prevalence of vaccinia over cowpox infections at the time when vaccines were established. Alternatively, both vaccinia and cowpox viruses may have been used to establish smallpox vaccines, but the

more severe reaction produced by cowpox and the choice of vaccine strains with lower pathogenicity over the years may have selected vaccinia as the 20th century smallpox vaccines [4].

CONCLUDING REMARKS

The ability of viruses to replicate and survive in an immunocompetent host depends on the extent to which the virus counteracts the activity of cells and molecules of the imunune system. Poxviruses encode a broad spectrum of proteins that counteract the host immune response to infection. These virus-encoded "weapons" may be indicators of the relative value of the various components of the immune system in combating virus infections. IFNs were discovered because of their ability to prevent virus infections and its key anti-viral activity is underscored by the numerous mechanisms that poxviruses, and other viruses, have evolved to counteract their activity [20, 56, 71].

Soluble proteins that mimic the extracellular binding domain of host cellular receptors are used by poxviruses to efficiently counteract the function of cytokines *in vivo*. A similar strategy, based on recombinant soluble versions of cytokine receptors, is being tested to block cytokine activities in different disease conditions and may represent important therapeutic agents in the future.

The proteins that viruses use to evade the host immune system were acquired during virus evolution in different hosts. Thus careful analysis of the interaction between the poxvirus cytokine receptors and their ligands will shed light on the evolutionary history of poxviruses. The broad species specificity of the orthopoxvirus IFN-γR is unique among IFN-γRs and suggests virus replication in several species during evolution and possible natural hosts. This is particularly important for vaccinia virus, a virus that was a successful vaccine for smallpox eradication but whose origin and natural host(s) are unknown.

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