p27, Ty-VLP, or TT (200 or 20 μg/ml) for 7 days, followed by culture without antigen for 8 days. The culture supernatants were assayed for the corresponding IgA or IgG by a modified ELISA. Microtiter plates were coated with antigen (p27, p27:Ty-VLP, Ty-VLP, or TT; 1 μg/ml). Culture supernatants were diluted 1:1 with RPMI 1640 medium before incubation. Bound antibody was detected with goat antibody to monkey IgA or IgG (Nordic Immunological Laboratory), followed by biotinylated rabbit antibody to goat IgG, horseradish peroxidase, and phenylenediamine dihydrochloride. The reaction was terminated with 2 M H_2SO_4. The results are expressed as mean (± SEM) absorbance at a wavelength of 492 nm, with the absorbance of the control culture (of $CD4^+$ cells, B cells, and macrophages but without antigen) subtracted. The results are presented only for stimulation with p27 (200 ng/ml), Ty-VLP (200 ng/ml), or TT (20 ng/ml) and tested against the corresponding antigen. Stimulation with one antigen and tested against another antigen did not show an absorbance greater than 0.15 unit. A control macaque immunized by the V-O route with Ty-VLP/CTB and boosted by IM immunization did not yield anti-p27.

15. Mononuclear cells were separated (*12*) and cultured without antigen and with p27, p27:Ty-VLP, Ty-VLP, CTB, R20, and concanavalin A (1, 10, and 20 μg/ml) in 96-well round-bottomed plates (Costar) containing RPMI 1640 medium (Gibco) supplemented with penicillin (100 units/ml; Sigma), streptomycin (100 μg/ml; Sigma), L-glutamine (2 mmol/liter; Sigma), and 10% autologous serum for 4 days. The cultures were then pulsed with 0.5 μCi of [^{3}H]thymidine for 4 hours. The cells were then harvested on filter paper discs, and the [^{3}H]thymidine uptake was determined by scintillation counting. The results were expressed as stimulation indices (ratio of counts with and without antigen) and as counts per minute for cultures stimulated with p27 (10 μg/ml); those stimulated with p27:Ty-VLP gave similar results. All cultures yielded high stimulation indices and counts with concanavalin A, and no significant counts were found with CTB or R20. The mucosal route of immunization did not elicit a rise in [^{3}H]thymidine uptake when the cells were stimulated with Ty-VLP. However, after IM administration of the immunogens, moderate responses were elicited by stimulation with Ty-VLP.
16. IgA antibodies to p27 and a control random peptide of 20 amino acids (R20) was determined by ELISA. Plates coated with antigen (at 1 μg/ml) were incubated with doubling dilutions of test samples. Bound antibody was detected by incubation with rabbit IgG to monkey IgA at 8 μg/ml or monkey IgG at 2 μg/ml (Nordic Immunological Laboratory), followed by affinity-purified goat antibody to rabbit IgG conjugated to alkaline phosphatase (Sigma Fine Chemicals) and *p*-nitrophenylphosphate disodium (Sigma Diagnostics). The reaction was terminated with 3 M NaOH, and the absorbance measured at a wavelength of 405 nm. Results are expressed as the reciprocal of the lowest dilution that gave an absorbance of 0.15 units above the background sample. The reproducibility of the ELISA after four repeated assays of the same vaginal fluid sample for IgA and IgG was within one dilution. The results with R20 were negative.
17. The construction of hybrid virus-like particles containing the SIV p27 sequence of isolate 32H of $SIVmac_{251}$ fused to the p1 protein of Ty has been described [N. R. Burns, J. E. M. Gilmour, S. M. Kingsman, A. J. Kingsman, S. E. Adams, *J. Mol. Biol.* **216**, 207 (1990); N. R. Burns, in *Methods in Molecular Biology*, M. Collins, Ed. (Humana, Clifton, NJ, 1991), vol. 8, p. 277]. The SIV gag p27 gene was derived from the clone pNIBSCI, and the p27:Ty-VLP and control Ty-VLP were purified from yeast extracts [N. Almond *et al.*, *J. Virol. Methods* **28**, 301 (1990)]. Nonparticulate p27 was prepared by cleavage from the p27:Ty-VLP and further purified by ion exchange chromatography. The absence of any Ty protein in the p27 preparation was confirmed by protein immunoblotting. The recombinant antigens were covalently linked to CTB (Sigma) at a ratio of 1:1 with SPDP (*N*-succinimidyl-3,2-pyridyl dithiopropionate) [C. Czerkinsky *et al.*, *Infect. Immun.* **57**, 1072 (1989)]. Six rhesus macaques received two oral, followed by three vaginal immunizations (O-V) of p27:Ty-VLP/CTB (n = 3), p27:Ty-VLP without CTB (n = 1), p27/CTB without Ty-VLP (n = 1), or p27 (n = 1) at monthly intervals. Three rhesus macaques and one cynomolgus macaque received two vaginal, followed by three oral (V-O) monthly immunizations of p27:Ty-VLP/CTB, and one control rhesus macaque received Ty-VLP/CTB. Topical vaginal administration of 200 μg of p27:Ty-VLP/CTB (or Ty-VLP/CTB) was carried out with soft, lubricated pediatric nasogastric tubes. Oral administration was performed by intragastric intubation of gelatin-coated capsules, containing 500 μg of the vaccine and 400 μl of cholera vibrio (Institut Merieux, Lyon, France) in the presence of sodium bicarbonate. One month after the last mucosal administration, all macaques were challenged by IM immunization with 200 μg of the preparation that we used to immunize that animal and mixed with aluminium hydroxide (AluGel, Uniscience, London, U.K.), except for the p27/CTB-immunized animal, which received 130 μg of p27/CTB in AluGel. Blood was collected from the femoral vessels, and the serum was separated. Vaginal and rectal washings were collected atraumatically with the aid of pediatric naso-gastric tubes that were flexible and lubricated. Samples were collected before and approximately 1 month after each immunization; the results shown are from samples taken approximately a month after the given immunization schedule. All procedures in the macaques were carried out after sedation with I M ketamine hydrochloride (10 mg/kg; Parke-Davis Veterinary).

18. We thank N. Graff and N. Burns (British Biotechnology, Ltd.) for preparing the SIV antigens and P. Kitchin and N. Almond (National Institute of Biological Standards and Control) for supplying plasmid pNIBSCI, containing the p27 gene we used to construct the p27:Ty-VLP. Supported by the Medical Research Council AIDS Directed Programme.

26 June 1992; accepted 21 September 1992

Encoding of a Homolog of the IFN-γ Receptor by Myxoma Virus

Chris Upton, Karen Mossman, Grant McFadden*

Many poxvirus-encoded virulence factors have been identified as proteins that are secreted from infected cells. The major secreted protein (37 kilodaltons) from cells infected with myxoma virus is encoded by the M-T7 open reading frame. This protein has significant sequence similarity to the human and mouse receptors for interferon-gamma (IFN-γ). Furthermore, the myxoma M-T7 protein specifically binds rabbit IFN-γ and inhibits the biological activity of extracellular IFN-γ, one of the key regulatory cytokines in the host immune response against viral infections.

The poxviruses comprise a family of large, complex, and relatively autonomous double-stranded DNA viruses that replicate in the cytoplasm of host eukaryotic cells (*1*). Although they have been shown to infect mammals, birds, reptiles, and insects, the severity of the disease produced by different poxviruses varies greatly (*2, 3*). In general, poxvirus genomes are organized with essential genes clustered in the center, whereas virulence markers that govern pathogenesis tend to map toward the termini of the genome (*4*). A number of these virulence markers are secreted proteins that enhance the ability of the virus to propagate in its natural host but have only a minor effect, if any, in cultured cells. These "virokines" include epidermal growth factor–like growth factors, a complement binding protein, and serine protease inhibitors (*2, 5*). Shope fibroma virus (SFV) encodes a secreted protein that has significant sequence similarity to the host tumor necrosis factor (TNF) receptor (*6, 7*); the deletion of the homolog of this gene in the myxoma virus reduces the virulence of this virus considerably (*8*). Wild-type myxoma virus is extremely virulent in adult domestic rabbits and has apparently evolved multiple mechanisms to combat the host immune response to the infection (*9*). It therefore provides a useful model to study the general mechanisms of poxvirus virulence, a greater understanding of which is required for the development of safer poxvirus vaccines for humans and animals (*10, 11*). The self-defense weapons that are encoded by such highly virulent viral pathogens (*12*) may also be important indicators of the relative value of the various components of the immune system in combating such disease agents in vivo.

Labeling studies have shown that a number of secreted proteins are specific to myxoma infected cells (*13*). Figure 1A shows proteins secreted at early and late (after viral DNA replication begins) times during an infection of BGMK cells by myxoma virus (strain Lausanne) in comparison to mock-infected cells. The most prominent [^{35}S]methionine + cysteine protein (arrow in Fig. 1A) was observed at both early (lane E) and late (lane L) times of infection. After concentration, the same protein was readily detected as the predominant band in Coomassie blue–stained polyacrylamide gels and migrated with an apparent size of ap-

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

*To whom correspondence should be addressed.

proximately 37 kD (Fig. 1B). This protein species was synthesized and secreted maximally between 1 and 4 hours after infection but continued to be made at later times and in greater quantities than any other secreted viral protein.

To identify this protein, we infected 10^9 BGMK cells with myxoma virus at a multiplicity of infection of 10 (in the absence of serum) and secreted proteins were collected during the early stages of infection (1 to 5 hours). After partial purification by ammonium sulfate precipitation, the proteins were subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE), blotted to polyvinylidine difluoride membrane (Millipore), and stained by Coomassie blue. The 37-kD band was excised, and NH_2-terminal analysis was done with an ABI 470A Protein Sequencer (Applied Biosystems Inc., Foster City, California). A sequence of 21 NH_2-terminal amino acids was obtained (Val-Arg-Leu-Thr-Ser-Tyr-Asp-Leu-Asn-Thr-Phe-Val-Thr-Trp-Gln-Asp-Asp-Gly-Tyr-Thr-Tyr) that had no counterpart in the current Protein Information Resource (PIR) database but scored a strong match in our catalog of poxvirus protein sequences. A total of 17 of the 21 amino acids (with no gaps added) were identical to a region in an open reading frame (ORF) designated S-T7 from the terminal inverted repeats (TIRs) of SFV, a Leporipoxvirus closely related to myxoma virus (*14, 15*). The position of the matching amino acid sequence in S-T7, adjacent to a predicted NH_2-terminal signal sequence, indicated that this SFV ORF is a closely related homolog of the abundantly secreted 37-kD myxoma virus protein.

To identify the myxoma counterpart to S-T7, we cloned and sequenced (the DNA sequence has been submitted to GenBank with accession number M81919) the equivalent region of the myxoma virus TIR (*16*), contained within a Sst I–Bam HI subfragment of the myxoma Bam HI K fragment (Fig. 2A). The identified M-T7 ORF is 263 amino acids long (Fig. 2B). The secreted protein sequence starts at residue 19 and has a calculated mass of 28.0 kD, 9.0 kD less than that observed with PAGE analysis. The 21–amino acid sequence identified by NH_2-terminal sequencing of the secreted myxoma protein is in complete agreement with the translated DNA sequence and is found adjacent to a consensus NH_2-terminal signal sequence (Fig. 2B). The mechanism for the extremely high rate of M-T7 synthesis and secretion is unknown. However, the rate of transcription of the M-T7 gene would be predicted to be high because the DNA sequence immediately 5′ of the gene is very similar to that identified as being optimal for early poxvirus gene expression (*17*) (Fig. 2B). In addition, the M-T7 protein is still efficiently synthesized and secreted at late times of infection (Fig. 1A), when the transcription of most early genes is switched off, which suggests that either the mRNA is unusually stable or the gene is also regulated by a late viral promoter.

The PIR database was searched for similarities to the M-T7 and S-T7 ORFs by means of NW_Align (*18*). This analysis revealed a low but significant similarity between both the M-T7 and S-T7 ORFs and the ligand binding domains of the human (*19*) and mouse (*20*) interferon-gamma receptors (IFN-γRs). An alignment of the poxviral ORFs (M-T7 and S-T7) and the mammalian IFN-γRs (human and mouse) is shown in Fig. 3, together with a previously unidentified ORF from vaccinia virus (*21*). The three poxvirus ORFs are considerably shorter than mammalian IFN-γRs and possess neither hydrophobic membrane-spanning domains nor counterparts of the cytoplasmic signaling domains. Similarity between the viral and cellular proteins is confined to the extracellular domain of the IFN-γRs, a situation similar to the homology of the myxoma/SFV T2 proteins with the ligand binding domain of the TNF receptor (*6–8*). Because a characteristic feature of many cytokine cellular receptors is the use of Cys-Cys bonds to maintain the structure of extracellular ligand binding domains (*22*), it

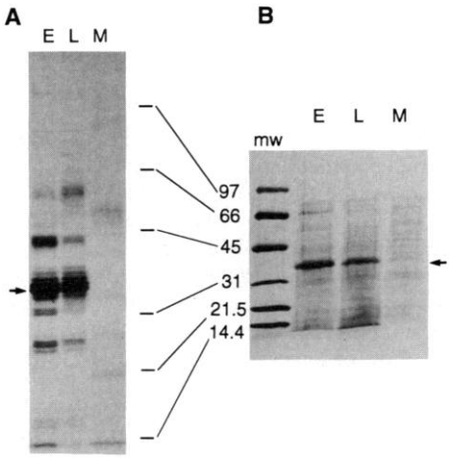

Fig. 1. Secretion of proteins from myxoma virus–infected cells. (**A**) Autoradiogram of [^{35}S]methionine + cysteine proteins secreted from cells infected with myxoma virus. E, early infection; L, late infection; and M, mock infection. At 0 hours, 4 × 10^7 BGMK cells were infected with wild-type myxoma virus that contained a β-galactosidase marker inserted in an intergenic position [strain of Lausanne designated myx-lac; multiplicity of infection (MOI) of 30] in 5 ml of medium [Dulbecco's modified Eagle's medium (DMEM) + 10% newborn calf serum]; after 1 hour, the cells were washed three times with phosphate-buffered saline (PBS) to remove serum and incubated with 200 μCi of [^{35}S]methionine + cysteine (Translabel, ICN) in 12 ml of methionine-deficient medium (Gibco); after 2 hours, the cells were washed three times with PBS and incubated in 12 ml of DMEM (no serum); after 5 hours, the medium was collected (early sample) and replaced with fresh medium; after 6 hours, the cells were washed and labeled as before; after 7 hours, the cells were again washed three times with PBS and incubated in 12 ml of DMEM (no serum); and after 16 hours, the medium was collected (late sample). Cells were also mock-infected and similarly labeled as a control for secretion of cellular proteins. Samples were centrifuged at 10,000*g* for 1 hour to remove cellular debris and concentrated 12-fold with the use of Centriprep10 filters (Amicon). Twenty-microliter samples were added to the loading buffer and electrophoresed in a 5 to 15% SDS–acrylamide gel. The gel was dried on Whatman 3MM paper and exposed to x-ray film. Arrow indicates the most prominent [^{35}S]methionine + cysteine protein. (**B**) Total proteins secreted from cells infected with myxoma virus. E, L, and M are as in (A); mw, molecular weight standards (Bio-Rad). At 0 hours, 3 × 10^8 BGMK cells (in roller bottles) were infected (or mock-infected) with myxoma virus (MOI of 10) in 10 ml of medium; after 2 hours (E and M), the cells were washed three times with PBS and incubated in 12 ml of DMEM (no serum); after 6 hours, E and M samples were collected, and virus-infected cells were washed once with PBS and re-incubated with 12 ml of DMEM (no serum); after 12 hours, the late sample was collected. Samples were centrifuged at 12,000*g* for 1 hour to remove debris. Solid ammonium sulfate was added (to 80%) to precipitate the majority of proteins. Pelleted proteins were resuspended in 300 μl of sodium phosphate buffer (pH 7), and 3-μl samples were electrophoresed in a 4 to 15% precast SDS–acrylamide gel (Bio-Rad) and stained with Coomassie blue. Arrow refers to a 37-kD protein analyzed by NH_2-terminal sequencing. Molecular size markers are indicated in kilodaltons.

Table 1. Myxoma T7 protein neutralizes the ability of rabbit IFN-γ to induce the antiviral state in RK13 cells. Cell monolayers (6 × 10^5 cells) were pretreated in 1 ml of medium that contained 10% newborn calf serum with different concentrations of recombinant rabbit IFN-γ alone or in combination with appropriate dilutions of T7-containing myxoma supernatants. After 16 hours at 37°C, the cells were washed and adsorbed with approximately 100 infectious units of VSV; plaques were counted after a further 24 hours. The data are expressed as the percent of viral plaques produced after pretreatment with the indicated concentrations of IFN-γ compared to identical monolayers infected in the absence of IFN-γ. T7 supernatant alone had no effect on VSV plaquing efficiency but consistently reduced the antiviral activity of IFN-γ at all concentrations tested. ND, not determined.

T7 protein (μg/ml)	Virus yield [% of uninhibited control in relation to the concentration of IFN-γ (×35 pg/ml)]					
	10^5	10^4	10^3	10^2	10^1	1
5	28	78	100	100	100	100
1	ND	45	85	100	100	100
0.1	ND	44	54	85	100	100
None	0	0	16	36.5	61	85

is notable that all eight cysteines are conserved in the extracellular domain of the IFN-γRs and S-T7/M-T7 (six are conserved in the vaccinia virus ORF). Twenty percent of amino acids in the extracellular domain of the mature IFN-γRs are identical in four of the five proteins, and numerous residue positions have highly conserved amino acids.

To test the hypothesis that M-T7 is a soluble viral counterpart of the host IFN-γR, we performed cross-linking experiments. ^{32}P-labeled rabbit IFN-γ was incubated with the secreted proteins from mock- or myxoma virus–infected cells, in the absence or presence of excess unlabeled IFN-γ, and the protein-to-protein complexes were cross-linked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Fig. 4). A labeled complex, with an apparent molecular weight of 51 kD, could be observed in the presence of virus-specific proteins (Fig. 4B, lane 5) but not when the secreted proteins from mock-infected cells were used (Fig. 4B, lane 3). This labeled complex was almost com-

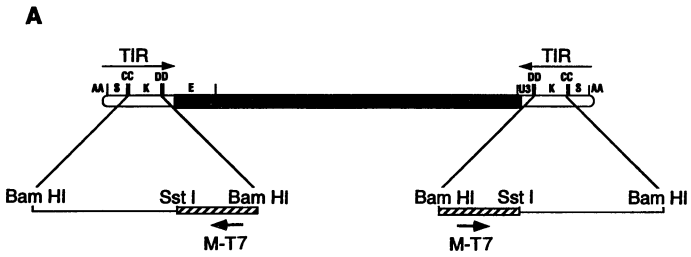

Fig. 2. Cloning and sequencing of myxoma virus ORF M-T7. (**A**) The myxoma virus genome is a linear double-stranded DNA of approximately 162 kb with hairpin termini (*16*). The TIRs of 11.5 kb are shown as open boxes and the central unique region (not to scale) is shown as a black bar. Only Bam HI fragments that span the TIRs are indicated. A 1.9-kb Sst I–Bam HI subfragment (shown as a striped bar) of the 2 M Bam HI K fragment was cloned into Bluescript vectors (Stratagene) and sequenced completely in both orientations with the use of single-stranded templates with dideoxynucleotides and either ^{35}S-labeled deoxyadenosine triphosphate with Sequenase (USB, Cleveland, Ohio) or an ABI 373A DNA Sequencer with Taq cycling. (**B**) The sequence of the complete Sst I–Bam HI fragment has been submitted to GenBank with accession number M81919; only the M-T7 ORF and immediate flanking sequences are shown here. The 21 amino acids identified by NH_2^*-terminal sequencing of the secreted polypeptide (italicized) and a consensus sequence (underscored) for a strong early poxvirus promoter (nucleotides 61 through 75) are highlighted (*33*). The termination codon (—>>***) of the 5′ ORF (M-T8) and the initiating codon (Met—>>) of the 3′ ORF (M-T6) are indicated.

B

```
1   GTCACTTCGC CACCATGAAC GCGGCGTACG CCACCTACCT CGAGCTGTAG AAACGTTTTT ATAACTGAAA
                                                    —>>***

71  AAGTATCCTA AAAATAGAGT AATACTCAAG ATG GAC GGG AGA CTG GTG TTT CTC CTC
                                     Met Asp Gly Arg Leu Val Phe Leu Leu

128 GCG TCG CTC GCT ATC GTC TCC GAC GCC GTA CGC CTT ACG TCC TAC GAC TTA AAC
    Ala Ser Leu Ala Ile Val Ser Asp Ala Val Arg Leu Thr Ser Tyr Asp Leu Asn

182 ACA TTC GTT ACG TGG CAA GAC GAT GGA TAC ACC TAC AAC GTC AGT ATT AAA CCG
    Thr Phe Val Thr Trp Gln Asp Asp Gly Tyr Thr Tyr Asn Val Ser Ile Lys Pro

236 TAT ACG ACG GCT ACG TGG ATC AAT GTG TGT GAA TGG GCG TCT TCT AGC TGC AAC
    Tyr Thr Thr Ala Thr Trp Ile Asn Val Cys Glu Trp Ala Ser Ser Ser Cys Asn

290 GTA TCT CTC GCC CTA CAA TAC GAT TTG GAC GTC GTG TCT TGG GCC AGA CTG ACC
    Val Ser Leu Ala Leu Gln Tyr Asp Leu Asp Val Val Ser Trp Ala Arg Leu Thr

344 CGG GTT GGT GGG TAC ACA GAA TAC AGT CTG GAA CCG ACG TGT GCC GTG GCT CGG
    Arg Val Gly Gly Tyr Thr Glu Tyr Ser Leu Glu Pro Thr Cys Ala Val Ala Arg

398 TTC TCT CCA CCG GAG GTA CAA CTC GTA AGA ACA GGT ACC AGC GTA GAA GTC TTA
    Phe Ser Pro Pro Glu Val Gln Leu Val Arg Thr Gly Thr Ser Val Glu Val Leu

452 GTT AGA CAC CCC GTC GTG TAT CTA CGG GGG CAG GAA GTG TCC GTC TAC GGA CAT
    Val Arg His Pro Val Val Tyr Leu Arg Gly Gln Glu Val Ser Val Tyr Gly His

506 TCA TTC TGC GAC TAC GAC TTC GGG TAT AAA ACG ATC TTC CTG TTC TCG AAG AAT
    Ser Phe Cys Asp Tyr Asp Phe Gly Tyr Lys Thr Ile Phe Leu Phe Ser Lys Asn

560 AAA CGA GCG GAG TAC GTC GTA CCC GGC CGA TAT TGC GAC AAC GTA GAG TGT CGT
    Lys Arg Ala Glu Tyr Val Val Pro Gly Arg Tyr Cys Asp Asn Val Glu Cys Arg

614 TTC TCC ATC GAT TCC CAA GAA AGT GTA TGT GCT ACG GCG GTT CTT ACG TAC GGT
    Phe Ser Ile Asp Ser Gln Glu Ser Val Cys Ala Thr Ala Val Leu Thr Tyr Gly

668 GAC AGT TAT CGT TCC GAG GCG GGT GTG GAG GTC TGC GTT CCC GAA CTC GCG AAG
    Asp Ser Tyr Arg Ser Glu Ala Gly Val Glu Val Cys Val Pro Glu Leu Ala Lys

722 AGA GAA GTC AGT CCC TAC ATC GTG AAA AAG TCG TCC GAC CTG GAA TAC GTC AAA
    Arg Glu Val Ser Pro Tyr Ile Val Lys Lys Ser Ser Asp Leu Glu Tyr Val Lys

776 CGT GCC ATA CAC AAC GAA TAC CGA CTC GAC ACC TCC TCC GAG GGA CGC AGA TTG
    Arg Ala Ile His Asn Glu Tyr Arg Leu Asp Thr Ser Ser Glu Gly Arg Arg Leu

830 GAG GAA CTG TAT CTA ACG GTC GCC TCC ATG TTT GAA CGT CTC GTG GAA GAT GTC
    Glu Glu Leu Tyr Leu Thr Val Ala Ser Met Phe Glu Arg Leu Val Glu Asp Val

884 TTC GAA TAATCGAAAT ATAAATAATG TAGTTTTTGT ATCGGAATCA TGGAACGTAC CCTGGTAAGT
    Phe Glu ***                                          M et —>>

950 TTCTTGGACA GCGGTACCAT GAGCGACATC ACCCTCGTCG
```

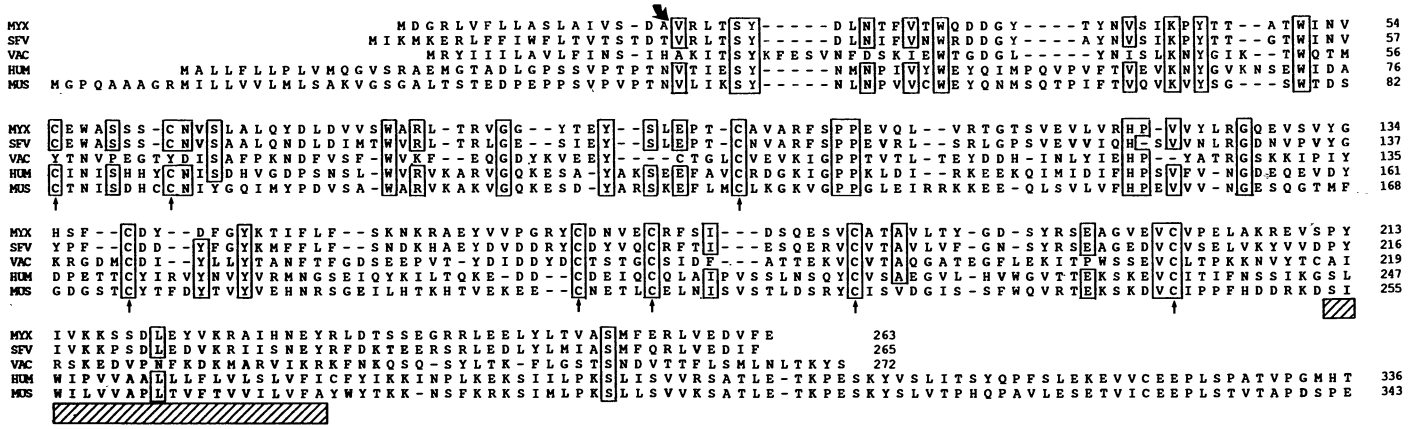

Fig. 3. Alignment of poxviral proteins with human and mouse IFNγRs (*33*). MYX, myxoma virus M-T7; SFV, Shope fibroma virus S-T7 (*15*); VAC, vaccinia virus B8R (strain Copenhagen) (*21*); HUM, human IFN-γR (*19*) (full length is 489 amino acids); MUS, mouse IFN-γR (*20*) (full length is 477 amino acids). Amino acids with at least 80% identity between the five sequences have been boxed. The large arrow indicates the NH_2-terminal residue of the mature secreted myxoma M-T7 protein. Small arrows show the eight conserved cysteines in the extracellular domains of the mouse and human IFN-γRs. A striped box represents the predicted transmembrane domains of the mouse and human IFN-γRs, which have no significant homology with the viral proteins.

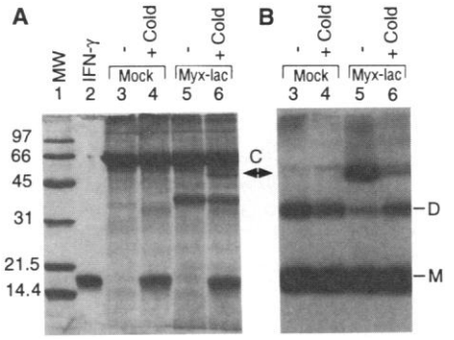

Fig. 4. Cross-linking of rabbit IFN-γ to the M-T7 protein. Rabbit IFN-γ was labeled by phosphorylation (*34*) with adenosine 3′,5′-monophosphate–dependent protein kinase (Sigma) and [γ-^{32}P]adenosine triphosphate (ATP) (ICN). Labeled IFN-γ (1.4×10^4 cpm/ng) was separated from [γ-^{32}P]ATP with the use of a 2-ml Sephadex G-25 column (Pharmacia, Uppsala, Sweden). Secreted protein (5 μl) (Fig. 1B) was incubated with 40 ng of [^{32}P]IFN-γ with or without 100-fold excess of unlabeled (cold) competitor IFN-γ for 2 hours at 22°C; cross-linking was performed by the addition of EDC (Sigma) to 40 mM (final concentration) at 22°C for 30 min and quenched by the addition of one-tenth volume 1 M tris (pH 7.5). The mixtures were centrifuged at 13,000*g* for 15 min at 4°C, and the resulting supernatants were added to loading buffer, boiled, and subjected to SDS-PAGE in a 15% gel. (**A**) Coomassie-stained gel. Lane 1, molecular weight (MW) markers; lane 2, recombinant rabbit IFN-γ; lanes 3 through 6, binding of secreted protein extracts (Mock, mock-infected cells; Myx-lac, early proteins secreted from cells infected with myx-lac virus) (Fig. 1B) to [^{32}P]IFN-γ in the presence or absence of excess unlabeled IFN-γ. The 66-kD protein band seen in lanes 3 through 6 is bovine serum albumin, which was added to the [^{32}P]IFN-γ labeling mixture as part of the stop buffer. (**B**) Autoradiogram of lanes 3 through 6 of gel shown in (A). Monomers and dimers of IFN-γ are indicated by M and D, respectively. C indicates the M-T7/IFN-γ complex visible by Coomassie staining [lane 6 in (A)] and autoradiography [lane 5 in (B)]. Molecular size markers are indicated to the left in kilodaltons.

pletely competed out upon the addition of 100-fold excess unlabeled IFN-γ (Fig. 4B, lane 6). However, this was replaced by an abundance of unlabeled complex that is clearly visible by Coomassie staining (Fig. 4A, lane 6) because M-T7 (Fig. 4A, lanes 5 and 6; 37 kD) is the only protein in these preparations in sufficient quantity to account for the stoichiometry of the complex. Although rabbit IFN-γ is a noncovalent homodimer, only heterodimers of IFN-γ and M-T7 were detected in our experiments. This result may be due to the low efficiency with which EDC cross-links the dimer IFN-γ itself because after SDS-PAGE, only a small fraction of the [^{32}P]IFN-γ was observed as a cross-linked dimer (Fig. 4B). The mobility of the M-T7/IFN-γ complex in SDS-PAGE was close to the predicted sum of 54.5 kD (37 + 17.5 kD). Recombinant human and mouse IFN-γ also formed complexes with the T7 protein but with less affinity.

Several biological activities can be measured for IFN-γ, one of the most sensitive being the induction of the antiviral state in which cells pretreated with IFN-γ become resistant to subsequent vesicular stomatitis virus (VSV) infection (*23*). In this assay, T7-containing myxoma supernatants were extremely efficient in abrogating the ability of rabbit IFN-γ to induce resistance to VSV in rabbit RK_{13} cells (Table 1). Because all effects of IFN-γ are believed to initiate with the binding of the cytokine to its cognate cell-bound receptor, it is likely that the T7 protein would be equally able to block other biological activities of IFN-γ, such as macrophage activation and induction of MHC markers.

These data indicate that the M-T7 protein represents a soluble counterpart of the rabbit IFN-γR and is secreted from cells infected by myxoma virus to specifically bind and thereby block the activity of IFN-γ in the antiviral host immune response. Using similar experiments, we have also shown that the S-T7 gene product binds rabbit IFN-γ (*24*). Although soluble forms of the cellular IFN-γR have been reported (*25*), this represents the first instance of a viral protein being specifically targeted to counteract an interferon ligand. IFN-γ is a pleiotropic lymphokine having both regulatory and direct effects in the immune system (*26, 27*). It is primarily produced by activated T cells and natural killer cells in response to activation by antigen and exerts its effects by binding to a specific membrane-bound receptor (*28, 29*). As the principal activator of macrophages, IFN-γ has a major role in the process of inflammation (*30*). The importance of IFN-γ in combating poxviral infections has been demonstrated by research showing that immunodeficient mice recover from infection with vaccinia virus, which is normally lethal, if the virus expresses and secretes mouse IFN-γ (*31*). At least one other poxviral protein, K3L of vaccinia virus, has been shown to exert an anti-interferon effect but does so within the infected cell, which counteracts the effects of IFN-α/β downstream of the ligand-receptor binding process (*32*).

In view of the fact that the myxoma-encoded homolog of the TNF receptor is a bona fide viral virulence factor that shields virus-infected cells from the antiviral activity of TNF-α and TNF-β, we proposed the term "viroceptor" to describe virus-encoded homologs of cellular receptors for antiviral cytokines (*8*). Viroceptors thus function by intercepting the cellular immune response pathways dependent on the target cytokines, and the myxoma M-T7 protein is the first viral example of this strategy used against the interferon family.

REFERENCES AND NOTES

1. B. Moss, in *Virology*, B. N. Fields and D. M. Knipe, Eds. (Raven, New York, 1990), pp. 2079–2111.
2. R. M. L. Buller and G. J. Palumbo, *Microbiol. Rev.* **55**, 80 (1991).
3. P. C. Turner and R. W. Moyer, *Curr. Top. Microbiol. Immunol.* **163**, 125 (1990).
4. P. Traktman, *Cell* **62**, 621 (1990).
5. G. J. Kotwal and B. Moss, *Nature* **335**, 176 (1988).
6. C. A. Smith *et al.*, *Science* **248**, 1019 (1990).
7. C. A. Smith *et al.*, *Biochem. Biophys. Res. Commun.* **176**, 335 (1991).
8. C. Upton, J. L. Macen, M. Schreiber, G. McFadden, *Virology* **184**, 370 (1991).
9. F. Fenner and F. N. Ratcliffe, *Myxomatosis* (Cambridge Univ. Press, London, 1965).
10. E. Paoletti, *Ann. N.Y. Acad. Sci.* **590**, 309 (1990).
11. B. Moss, *Science* **252**, 1662 (1991).
12. A. L. Notkins and M. B. A. Oldstone, Eds., *Concepts in Viral Pathogenesis III* (Springer-Verlag, New York, 1989).
13. T. H. Pennington, G. E. Carnegie, S. R. Alcock, *J. Gen. Virol.* **61**, 115 (1982).
14. A. Wills, A. M. DeLange, C. Gregson, C. Macaulay, G. McFadden, *Virology* **130**, 403 (1983).
15. C. Upton and G. McFadden, *ibid.* **152**, 308 (1986).
16. R. J. Russell and S. J. Robbins, *ibid.* **170**, 147 (1989).
17. A. J. Davison and B. Moss, *J. Mol. Biol.* **210**, 749 (1989).
18. NW_Align is an alignment program option of the package SEQSEE, developed by D. Wishart and R. Boyko at the University of Alberta.
19. M. Aguet, Z. Demblic, G. Merlin, *Cell* **55**, 273 (1988).
20. C. S. Kumar *et al.*, *J. Biol. Chem.* **264**, 17939 (1989).
21. S. J. Goebel *et al.*, *Virology* **179**, 247 (1990).
22. S. K. Dower, C. A. Smith, L. S. Park, *J. Clin. Immunol.* **10**, 289 (1990).
23. F. Dorner, M. Scriba, R. Weil, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1981 (1973).
24. C. Upton and G. McFadden, unpublished results.
25. D. Novick, H. Engelmann, D. Wallach, M. Rubenstein, *J. Exp. Med.* **170**, 1409 (1989).
26. G. Trinchieri and B. Perussia, *Immunol. Today* **6**, 131 (1985).
27. C. E. Samuel, *Virology* **183**, 1 (1991).
28. J. A. Langer and S. Pestka, *Immunol. Today* **9**, 393 (1988).
29. A. P. G. M. Van Loon *et al.*, *J. Leukocyte Biol.* **49**, 462 (1991).
30. A. Billiau and R. Dijkmans, *Biochem. Pharmacol.* **40**, 1433 (1990).
31. M. R. J. Kohonen-Corish, N. J. C. King, C. E. Woodhams, I. A. Ramshaw, *Eur. J. Immunol.* **20**, 157 (1990).
32. E. Beattie, J. Tartaglia, E. Paoletti, *Virology* **183**, 419 (1991).
33. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
34. A. Rashidbaigi, H. Kung, S. Pestka, *J. Biol. Chem.* **260**, 8514 (1985).

35. We thank M. Carpenter for the amino acid sequencing, D. Tovell for VSV stocks, and J. Pimm, R. Whitford, and P. D'Obrenan for help with the DNA sequencing. Computer analysis was performed at the Molecular Mechanisms in Growth Control Computer Facility, University of Alberta. The SEQSEE program was developed in the laboratory of B. D. Sykes, supported by the Medical Research Council Group in Protein Function, University of Alberta. Recombinant rabbit IFN-γ was a gift from Genentech, CA. Funded by the National Cancer Institute of Canada. K.M. is supported by a Natural Sciences and Engineering Research Council of Canada studentship and by the Alberta Heritage Foundation for Medical Research (AHFMR). G.M. is a Medical Scientist of the AHFMR.

1 May 1992; accepted 9 September 1992