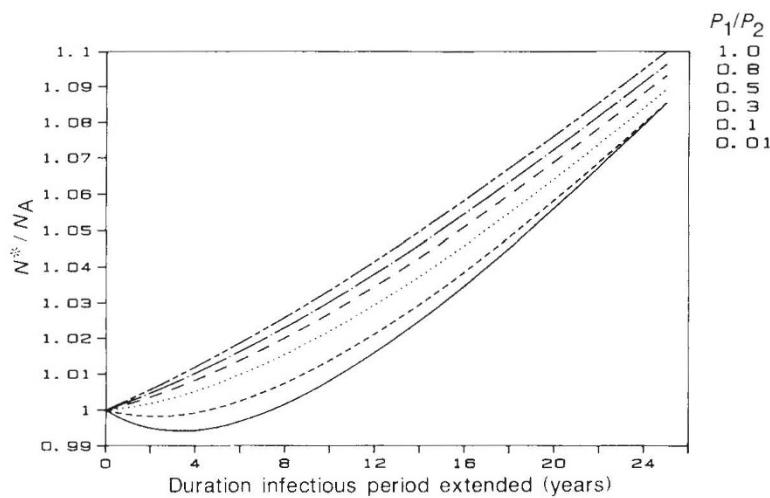


FIG. 3 The influence of targeting treatment to classes of high sexual activity. The graph records the population ratio N^*/N_A as a function of the degree to which treatment and/or vaccination extends the infectious (incubation) period of AIDS in infected persons ($1/(d+\mu) - 1/(v+\mu)$) and the degree to which treatment is targeted to classes of low (Y_1) or high (Y_2) sexual activity. The proportion, p_i , treated ($s=r=0, p \neq 0$) in the total population was set at 0.1 and the fraction receiving immunotherapy in each class (p_1 in the low-activity class and p_2 in the high-activity class) was varied. The different lines record the ratio p_1/p_2 with the top line denoting $p_1=p_2$ (no targeting) and the bottom line $p_1=0.01p_2$ (highly targeted) with intermediate levels in between. Note that a high degree of targeting makes matters worse (that is, ratio N^*/N_A becomes smaller) than without targeting. The model used to generate the calculations divided each susceptible and infected class on the basis of sexual activity, i (defined by the mean rate of acquisition of new sexual partners, c_i , in class i) where $dX_i/dt = (1-p_i)\gamma_i\mu N_0 - (c_i\lambda + \mu)X_i$; $dY_i/dt = c_i\lambda X_i - (\mu + v + r)Y_i$; $dV_{xi}/dt = p_i\gamma_i\mu N_0 - (c_i\lambda + \mu)V_{xi}$; and $dV_{yi}/dt = c_i\lambda V_{xi} + rY_i - (\mu + d)V_{yi}$. Here γ_i denotes the fraction of new recruits to the sexually active population that belong to class i . The term p_i is the fraction of class i receiving immunotherapy, which lengthens the incubation (infectious) period of AIDS (the calculations are here set to mimic immunotherapy as opposed to treatment with zidovudine). For this model, $N^* = N_0 \{1 - \lambda^* \Sigma [c_i \gamma_i \Psi_i / (c_i \lambda^* + \mu)]\}$. For a two sexual-activity-class model ($i=1\dots 2$), $\lambda^* =$



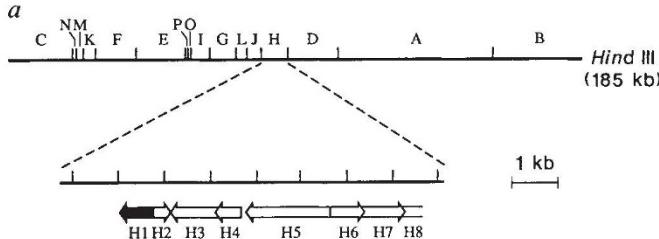
$\{-(\alpha b) \pm [(\alpha b)^2 - 4(\alpha a)(\alpha c)]^{1/2}\}/2\alpha a$, where $\alpha = \sigma_1\Psi_1c_2 + \sigma_2\Psi_2c_1 - cc_1c_2$, $\alpha b = \mu\beta[\sigma_1\phi_1c_2 + \sigma_2\phi_2c_1] + \mu[\sigma_1\Psi_1 + \sigma_2\Psi_2] - \mu c[c_1 + c_2]$, $\alpha c = \mu_2\beta \times (\sigma_1\phi_1 + \sigma_2\phi_2)$, given $c = \sum c_i$, $\sigma_i = c_i/\gamma_i$, $\phi_i = p_i/(\mu + d) + (1-p_i)/(\mu + v)$, $\Psi_i = dp_i/(\mu + d) + v(1-p_i)/(\mu + v)$. Parameter values: $p = 0.1$; $\beta c = 0.3 \text{ yr}^{-1}$; $\beta_1 = \beta_2$; $\gamma_1 = 0.8$; $\gamma_2 = 0.2$; $c_1 = 1.26 \text{ yr}^{-1}$; $c_2 = 9.96 \text{ yr}^{-1}$; $1/(\mu + v) = 8 \text{ yr}$; $1/\mu = 30 \text{ yr}$; $N_0 = 500,000.0$.

with the aim of changing behaviours that facilitate transmission to susceptible sexual partners. Calculations based on the assumption that treated persons reduce their sexual activity ($c_1 > c_2$) show that the degree to which activity must be reduced to negate the detrimental impact of community-wide drug application depends critically on the magnitudes of the other parameters. For R_0 values just >1 , a 50% reduction in c can eliminate the detrimental effects on the community. Second, the analyses emphasize the need in clinical trials (which test efficacy and toxicity) to assess the degree to which antiviral drug treatment or immunotherapy suppresses infectiousness.

Although our models are simple, and should be refined as more information becomes available, the main conclusions seem to be robust. In communities where the transmission rate of HIV is low, but sufficient for long-term persistence (R_0 not much larger than unity), treatment that lengthens the infectious period is likely to be able to increase overall transmission rates to more than counterbalance the greater longevity of infected individuals who are treated. The result is a perverse increase in the death rate from AIDS. This situation could apply to some heterosexual communities. When transmission rates are high (as in most communities using intravenous drugs and in some male homosexual populations), our models suggest that community-wide treatment is always beneficial for both individuals and the community (although not necessarily for linked communities, such as heterosexual networks coupled by bisexuals to male homosexual groups). By emphasizing the possible effects of zidovudine at the population-level, we hope to have drawn further attention to the need to improve counselling aimed at reducing high-risk behaviours. □

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RPTP	MEMEKEFECIDKAGNWAATYQDIRHEASDFPCRTIAKLPKNKNRNRYRDVSPFD	53		
HPTP	MEMEKEFECIDKSWSWAATYQDIRHEASDFPCRVAKLPKNKNRNRYRDVSPFD	53		
TPTP	MPTTIEREFEEELDTQRRWQPLYLEIRNESHDYPHRVAKFPEPNRNRNRYRDVSPYD	55		
VH1				
RPTP	HSRIKLHQEDNDYIWAISLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVV	108		
HPTP	HSRIKLHQEDNDYIWAISLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVV	108		
TPTP	HSRKVLQNAENDYIWAISLVDIEEAQRSYILTQGPLPNTCCIFWLWQKTKRAVV	110		
VH1	LULRSTGDMHKAKSPTIMTRVTNVNLGNYKNAAMDAPSSSEVREKLY VLNLTMDKYT	64		
RPTP	MUNRIMEKGSLKCAQYWPKKEEMVFFDDTNLKLTLISQSYTVRQLLELENLA	164		
HPTP	MUNRIVEKGSLKCAQYWPKKEEMVFFDDTNLKLTLISQSYTVRQLLELENLT	164		
TPTP	MUNRIVEKESVKCAQYWPTD DQEMLFKETGFSVLLSEDVRSYTVVHLQLQENIN	165		
VH1	LPNSNINIIHFLVDDTTTDISKYFDDVTAELSKCDQR NEPVVLVFGAAGV	114		
RPTP	TQEARMELHFRYTTWPDFGVPESSPASFLNELFKVRESGSLSLSPHEOPVVVHOSAGI	219		
HPTP	TQEARMELHFRYTTWPDFGVPESSPASFLNELFKVRESGSLSLSPHEOPVVVHOSAGI	219		
TPTP	TQEARMELHFRYTTWPDFGVPESSPASFLNELFKVRESGSLSLSPHEOPVVVHOSAGI	220		
VH1	MRSGAMIDAYLNSKNKESLPLMFLYVYHSMADLR GAFVENPSFKR	160		
RPTP	GRSGTCFLADTCLLMDKRKDPSVSD IKKVLLEMKFRMG LIQTADQLEFSYL	272		
HPTP	GRSGTCFLADTCLLMDKRKDPSVSD IKKVLLEMKFRMG LIQTADQLEFSYL	272		
TPTP	GRSGTCFLADTCLLMDKRKDPSVSD IKKVLLEMKFRMG LIQTADQLEFSYL	270		
VH1	QIIE KYVIDKN*	171		
RPTP	AVIEGAKFIMGDSSVQDQWKELSHEDLEPPPEHVPPPRPPKRTLEPHNGKCKEL	327		
HPTP	AVIEGAKFIMGDSSVQDQWKELSHEDLEPPPEHVPPPRPPKRTLEPHNGKCKEL	327		
TPTP	AVIEGAKFIMGDSSVQDQWKELSHEDLEPPPEHVPPPRPPKRTLEPHNGKCKEL	325		
RPTP	FSNHQWVSEESCEDEDILAREESRAPSIAVHSMS-SMSQDTEVRKRMVGGLQSA	381		
HPTP	FPNHQWVKEETQEDKDCPIEEKKGSPNLAAPYCGIESMSQDTEVRSRVVGGLRGA	382		
TPTP	TPGDRCTGLSSKMQDTMEENSESALRKRIEADRKTAAQKVQQMKQRLNEWERKRK	380		
RPTP	QASVPTEEELSPTEEEQKAHRPVHWKPFLVNVCATATALATGAYLCYRVCFH*	432		
HPTP	QASVPACKEPS LPEKDEDHALSYWKPFLVNMCVATVLTAGAYLCYRFLFNNT*	435		
TPTP	RWLYWQPILTKMGFMCSVILVGAFVGWTFLFFQQNAL*	415		

FIG. 1 a, Genomic map of vaccinia virus. The *Hind* III restriction map of vaccinia virus was adopted from Moss^{16,30}. The open reading frames of the *Hind* III H fragment are shown in detail. Filled arrow, open reading frame for VH1. b, Sequence alignment of VH1^{16,22} with rat brain PTP1 (RPTP)¹⁵, human placenta PTP-1B (HPTP)^{12,13} and T-cell PTP (TPTP)¹⁴. The residues that are completely conserved are indicated by a black background. METHODS. Sequences were aligned using a combination of the FASTA and BESTFIT²¹ programmes. For maximum alignment, gaps were introduced. *, end of sequence.

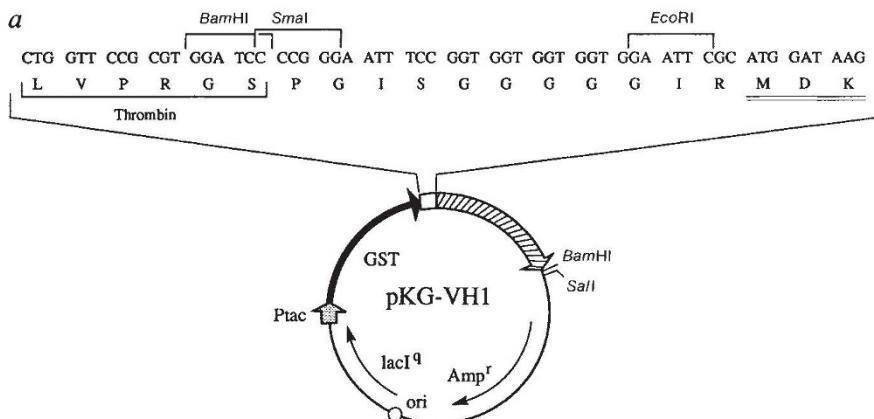
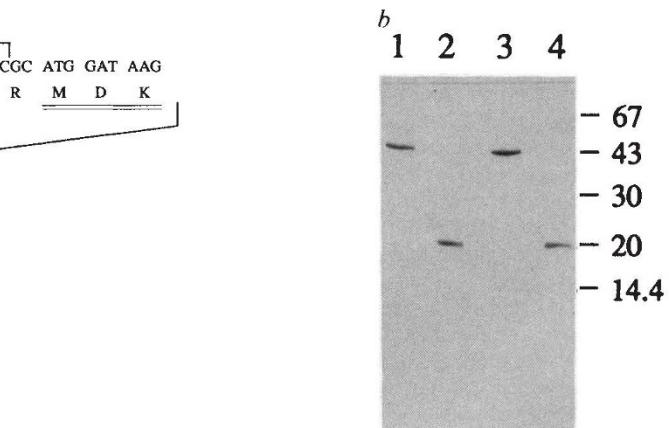


FIG. 2 Expression of recombinant vaccinia phosphatase. a, The plasmid pKG-VH1 was used to express VH1. Filled arrow, coding sequence for GST. Hatched arrow, coding sequence for VH1. Ptac, tac promoter. Open bar, thrombin cleavage site (cleavage occurs between the arginine and glycine residue) and the 'glycine kinker', with sequence information shown above the nucleotide sequence. The double underlined amino acid residues indicate the N terminus of VH1. b, SDS-PAGE of purified proteins. M_rs of standards (k) are shown on the right side of the gel. Lane 1, GST-VH1; lane 2, VH1; lane 3, GST-VH1 (C110S); lane 4, VH1 (C110S).

METHODS. Plasmid PH1 (refs 16, 22), which contains the open reading frame of VH1, was used as a template in polymerase chain reaction (PCR). Two primers were used in PCR of the *Vh1* gene. The 5' primer was (5'ATCCGATTCCGCATGATAAGAAAAGTTG) and the 3' primer was (5'CGTCACGGATCCAGACTTTAATTCTTATC). PCR was performed at 94 °C for 1 min., 45 °C for 2 min, and 72 °C for 2 min for 2 cycles and at 94 °C for 1 min, 50 °C for 2 min and 72 °C for 2 min for 30 additional cycles. The PCR product was



digested with EcoRI and BamHI and electrophoresed on an agarose gel. A fragment of ~0.5 kb was recovered and inserted into the EcoRI/BamHI-digested vector pT7-7 to produce pT7-VH1. A 0.5-kb EcoRI-SalI fragment (*Sal*I is located within the polylinker of pT7-7 adjacent to the 3' end of VH1) was isolated from pT7-VH1 and inserted into EcoRI/SalI-digested pGEX-KG to produce pKG-VH1. Plasmid pKG-VH1 was used to express VH1 as a fusion protein with GST. Expression and purification of VH1 was performed as described²³. Purified proteins were analysed by SDS-PAGE. Approximately 5 mg per litre of bacterial culture of GST-VH1 were routinely obtained. Site-directed mutagenesis was done by taking the EcoRI-BamHI fragment from pKG-VH1 and subcloning it into M13mp18. Codon TGT for Cys 110 was mutated to TCT for Ser using oligonucleotide-directed mutagenesis. The mutation was confirmed by DNA sequencing. The mutated DNA was subcloned into EcoRI/XbaI-digested pGEX-KG. Expression and purification of VH1 (C110S) mutant was carried out as previously described²³. After purification of VH1 and VH1 (C110S), glycerol was added to ~40%. The enzyme was stored at -20 °C.

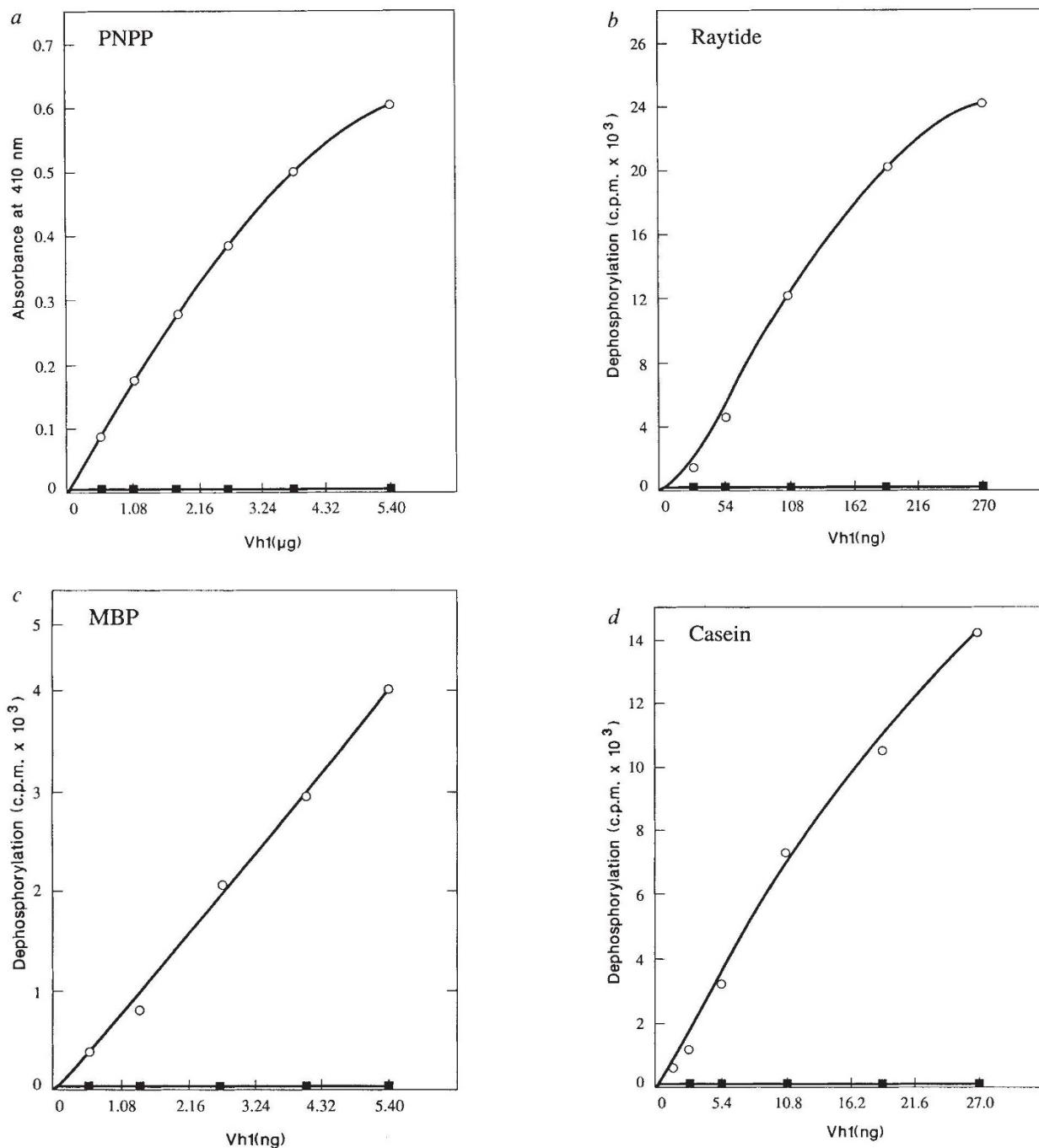


FIG. 3 Phosphatase activity of purified VH1 and VH1 (C110S). ○, VH1; ■, VH1 (C110S). *a*, Hydrolysis of *p*-nitrophenyl phosphate (PNPP). *b*, Dephosphorylation of Raytide. *c*, Dephosphorylation of myelin basic protein (MBP). *d*, Dephosphorylation of casein.

METHODS. Hydrolysis of PNPP was done in 200 μl of solution containing 50 mM imidazole, pH 7.5, 0.1% β-mercaptoethanol, 10 mM PNPP and enzyme at room temperature for 10 min. The reaction was stopped by addition of 800 μl 0.25 M NaOH. Absorbance at 410 nm was measured. Raytide was phosphorylated on tyrosine by p43^{v-abl} (Oncogene Science) as follows: 10 μg Raytide (Oncogene Science), 50 mM HEPES buffer, pH 7.5, 10 mM MgCl₂, 0.067% β-mercaptoethanol, 0.05 mM ATP including 300 μCi [γ -³²P]ATP, 4 U p43^{v-abl} kinase in a final volume of 60 μl. The reaction proceeded at 30 °C for 30 min and was stopped by addition of 240 μl 10% phosphoric acid. The sample was spotted onto two 1 × 1 cm P81 sheets of phosphocellulose paper and extensively washed with 0.5% phosphoric acid. Phosphorylated Raytide was eluted twice with 1 ml 500 mM (NH₄)₂CO₃, lyophilized and resuspended in 100 μl H₂O. Phosphorylation on bovine MBP (Sigma) was similar to that for Raytide, except that MBP (30 μg) was used instead of 10 μg Raytide in each phosphorylation reaction. Ice-cold trichloroacetic acid (TCA) (40 μl of a 50% solution) was used to precipitate MBP on ice for

30 min. After centrifugation, MBP was washed three times with 20% TCA and dissolved in 100 μl H₂O. Phosphorylation of casein was done using the catalytic subunit of protein kinase A. Casein, 200 μg, was phosphorylated in 200 μl solution containing 40 mM Tris, pH 7.5, 20 mM magnesium acetate, 0.2 mM ATP including 300 μCi [γ -³²P]ATP, and 10 μg catalytic subunit protein kinase A at 30 °C for 30 min. Phosphorylated casein was precipitated by addition of TCA to 20%, washed with 20% TCA to remove unincorporated [γ -³²P]ATP, and dissolved in 100 μl H₂O. Dephosphorylation was performed in 20 μl 50 mM imidazole, pH 7.5, 0.1% β-mercaptoethanol, purified enzyme and phosphorylated substrate, at room temperature for 10 min. Because of the low stoichiometry of phosphorylation on substrates, concentration referred to the actual phosphorylated substrate concentration. Phospho-Raytide or MBP (each at 10 nM) or 100 nM phosphocasein was used. Quantitation of dephosphorylation of Raytide was determined by a published method⁹. When MBP or casein was used as substrate, 20 μl of the dephosphorylation reaction were added to 10 μl BSA (10 mg ml⁻¹) and 180 μl 20% ice-cold TCA to stop the reaction and precipitate protein. ³²P in the soluble fraction was counted to measure the amount of dephosphorylation. Reactions without VH1 were used as the negative control.

surrounding the active site of receptor and non-receptor-like PTPases (GPIVVHCSAGVGRG in single-letter amino-acid code), we searched the National Biomedical Research Foundation data bank for other proteins having the conserved PTPase active-site sequence²¹. Numerous members of the PTPase family, the *Yersinia* PTPase and a protein encoded by an open reading frame in vaccinia virus were identified^{16,22}. The open reading frame in the vaccinia virus genome, designated *H1*, is shown in Fig. 1a. The protein expressed from the *H1* gene appears in the late stage of viral infection²² and its function is unknown.

Sequence alignment of the vaccinia *H1* gene product (VH1) with the non-receptor-like PTPases^{3,12-15} is shown in Fig. 1b. There is ~20% amino-acid identity with the PTPases. The VH1 protein is small (relative molecular mass 20,000 (M_r 20K)) compared with other non-receptor-like PTPases which have an M_r of ~50K. This may reflect efficient use of the compact virus genome. The stop codon in the *Vh1* gene is near the stop codon in the *Yersinia*¹⁷ and yeast PTPase (K.G., R. J. Deschenes, H. Qiu and J.E.D., unpublished results). It has been suggested that the mammalian PTPases have two domains, an N-terminal catalytic domain and a C-terminal domain important in regulation and/or targeting of the protein to the membrane¹⁵.

To determine whether VH1 possesses tyrosine phosphatase activity, the *Vh1* gene was isolated using the polymerase chain reaction. The 0.5-kilobase coding fragment of *Vh1* was subcloned into the vector pGEX-KG and expressed as a fusion protein with glutathione-S-transferase (GST) (Fig. 2a). The fusion protein, GST-VH2, was rapidly purified under non-denaturing conditions on a glutathione agarose affinity column²³. VH1 was cleaved from the fusion protein by thrombin. Both GST-VH1 and VH1 were obtained in a highly purified form as is evident from SDS-PAGE (Fig. 2b). The purified VH1 and GST-VH1 proteins were examined for their ability to dephosphorylate *p*-nitrophenyl phosphate, the phosphotyrosine containing peptide, Raytide, and myelin basic protein. The latter two were specifically phosphorylated on tyrosine using p43^{v-abl} kinase. VH1 hydrolyses *p*-nitrophenyl phosphate rapidly (Fig. 3a). Purified GST-VH1 is also active and has a specific activity comparable to VH1 (data not shown). The rates of reaction are dependent on the time of incubation and substrate concentration (data not shown). The hydrolysis of phosphate from phosphorylated myelin basic protein and Raytide are shown in Fig. 3b and c. Both substrates can be completely dephosphorylated by either VH1 or the corresponding fusion protein. We also examined casein, phosphorylated by the catalytic subunit of protein kinase A, as a substrate. VH1 was not expected to dephosphorylate Ser- or Thr-containing substrates as other PTPases show a specificity for tyrosine phosphate^{4,15,17}. Dephosphorylation of Ser-phosphorylated casein is shown in Fig. 3d. To verify that the phosphoamino acid residue in casein was not Tyr, phosphoaminoacid analysis was performed. Ser was the only residue phosphorylated in casein whereas phosphotyrosine was recovered from Raytide and myelin basic protein (data not shown). At 0.5 μ M substrate, Ser-phosphorylated casein was dephosphorylated by VH1 seven times more rapidly than Tyr-phosphorylated Raytide. Kinetic studies are underway to define the kinetic parameters of Ser- and Tyr-dephosphorylation.

Cys 215 in the rat brain PTPase and the corresponding Cys residues in the receptor-like PTPases and the *Yersinia* phosphatase are essential for catalytic activity^{9,17,24}. We used site-directed mutagenesis to alter Cys 110 in VH1 to Ser. The mutant VH1 (C110S) was expressed in the pGEX-KG vector and the fusion protein was obtained in a highly purified form (Fig. 2b). This protein was cleaved by thrombin, and the activities of the fusion protein and cleavage product were examined. Neither GST-VH1 (C110S) nor VH1 (C110S) displayed detectable activity towards phosphorylated Raytide, myelin basic protein, or casein (Fig. 3). This demonstrates the importance of Cys 110 in the hydrolysis of both Tyr and Ser phosphoproteins. The

hydrolysis of both substrates probably proceeds by a common catalytic mechanism using an essential Cys residue.

PTPases show a specificity for tyrosine phosphate hydrolysis and have a 'signature sequence' at their active site, namely HCXAGXXR. The mechanism of this family of phosphatases proceeds through a phospho-thiol intermediate involving the Cys in the signature sequence (K.G. and J.E.D., unpublished results). Type-2 Ser/Thr phosphatases also have residual tyrosine phosphatase activity²⁵⁻²⁸ which is dependent upon divalent cations. To verify that VH1 does not require a divalent cation for catalysis, VH1 activity was examined in the presence of EDTA. The Tyr or Ser phosphatase activity of VH1 is not inhibited by 10 mM EDTA. Okadaic acid, a Ser/Thr phosphatase inhibitor with 50% inhibitory concentration (IC_{50}) of 20 nM and 0.2 nM for type 1 and 2A enzymes respectively, was also tested²⁵. No inhibition of either Tyr or Ser phosphatase activity of VH1 was observed at concentrations of up to 1 μ M okadaic acid. In contrast, 1 mM sodium vanadate completely inhibits the activity of VH1.

Cohen and Cohen discovered a Ser/Thr protein phosphatase encoded by bacteriophage λ ²⁹, and suggested that the λ protein phosphatase may regulate production of viral RNA and protein. Although the function of the vaccinia phosphatase is unknown, it could have a pronounced effect upon tyrosine and/or serine phosphate content in cells infected with the virus. The vaccinia phosphatase may also play an important part in viral replication. In either case, it would seem that this virus, as well as pathogenic bacteria (*Yersinia*), probably exploit the phosphorylation-dephosphorylation signal transduction pathway by altering the extent of cellular protein phosphorylation. This may contribute to the pathogenic effects of these infections. The potential importance of the phosphatases in diseases caused by the family of pox viruses and the bubonic plague underscore the importance of further understanding events associated with the molecular pathogenesis of the PTPases. □

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