unlinked to PrP can also have a major influence on incubation times (*21, 22*). Because Tg(GSSPrP)174 mice were not inbred, being derived from (C57BL/6 × SJL)F2 fertilized oocytes (*23*), some of the variation may be due to modifier genes (*24, 25*).

The development of spontaneous spongiform neurodegeneration in Tg(GSSPrP)-174 mice establishes that a neurodegenerative process similar to a human disease can be genetically modeled in an animal. The argument that the leucine PrP variants in humans and Tg(GSSPrP)174 mice are potent susceptibility genes for a ubiquitous but unidentified pathogen remains a formal interpretation of our results (*26*). However, multiple lines of investigation on the physical and biological properties of the infectious agent consistently converge on PrP^{Sc} and fail to reveal additional essential components (*4*). Studies of neurodegeneration in Tg(GSSPrP) mice may provide new insights into the pathogenesis of some more common genetic and sporadic central nervous system degenerative disorders such as Huntington's and Alzheimer's diseases (*4, 24*).

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30. We thank D. Westaway and C. Mirenda for donating the *Prn-p*^{b} cosmid, R. Kochler for help in constructing the $MoGSSPrP_{Leu^{101}}$ ORF plasmid, M. Torchia and D. Rapp for care of the transgenic mice, L. Gallagher for document production assistance, and G. Carlson and C. Weissmann for helpful discussions. K.K.H. is the recipient of awards from the National Institutes of Health (CIDA–National Institute of Neurological and Communicative Diseases), Bernard Osher Philanthropic Fund, Culpeper Foundation, California Alzheimer's Disease Program, and Alzheimer's Disease and Related Disorders Association. Supported by NIH research grants AG02132, NS14069, NS22786, and AG08967; by the American Health Assistance Foundation; and by gifts from the Sherman Fairchild Foundation and National Medical Enterprises.

24 September 1990; accepted 29 October 1990

Broadly Neutralizing Antibodies Elicited by the Hypervariable Neutralizing Determinant of HIV-1

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The principal neutralizing determinant (PND) of human immunodeficiency virus (HIV)–1 resides within the V_3 loop of the envelope protein. Antibodies elicited by peptides of this region were able to neutralize diverse isolates. Serum from one of three animals immunized with the human T cell lymphoma virus (HTLV)–III_{MN} PND peptide, RP142, neutralized MN and the sequence-divergent HTLV-III_B isolate. Serum from one of three animals immunized with a 13–amino acid III_B PND peptide (RP337) also neutralized both of these isolates. Characterization of these sera revealed that the cross-neutralizing antibodies bound the amino acid sequence GlyProGlyArgAlaPhe (GPGRAF) that is present in both isolates. This sequence is frequently found in the PNDs analyzed in randomly selected HIV-1 isolates. Sera from two rabbits immunized with a peptide containing only the GPGRAF residues neutralized divergent isolates, including III_B and MN.

A LARGE NUMBER OF HIV-1 ENVELOPE reagents have been tested as immunogens to elicit neutralizing antibody. Commonly, resultant sera neutralize isolates related to the immunogen but not to divergent HIV-1 isolates. This isolate-restricted response is largely due to recognition of a variable domain of the virus envelope referred to as the V_3 loop or the principal neutralizing determinant (PND). It is contained within a hypervariable, 36–amino acid, disulfide-crosslinked loop in the large envelope subunit (amino acids 303 to 338) (*1, 2*). Synthetic peptides consisting of sequences within this region induce isolate-restricted neutralizing antibody (*1–6*) and monoclonal antibodies that bind the PND neutralize virus infectivity (*7–9*). The sequences of two peptides corresponding to the PND of the III_B isolate (RP337) and the MN isolate (RP142) are shown in Table 1. Three guinea pigs were immunized separately with each of these peptides, and the immune sera were assayed for the ability to neutralize the III_B and MN isolates and to prevent fusion of uninfected CD4 cells with cells infected with these isolates (Table 2). As expected on the basis of experience with these immunogens, immune sera from all of the animals immunized with RP142 neutralized and inhibited fusion of the MN isolate. Surprisingly, sera from one of these (animal g89) also neutralized and inhibited fusion of the III_B isolate. Similarly, one of

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Table 1. Sequences of the peptides of the principal neutralizing domain that were used are shown. A cysteine was added to the COOH-terminus of each peptide to allow conjugation to KLH (*20*). Dashes (–) indicate identity with the RP135 sequence. The numbers above the RP135 sequence denote the positions of the peptide in the III_B envelope protein (*21*).

Peptide	Viral isolate	Sequence
		307 331
RP135	III_B	NNTRKSIRIQRGPGRAFVTIGK IG(C)
RP337	III_B	----------(C)
RP142	MN	Y-K--R-H- ------Y-TKNI--(C)
RP111	III_B	$(IQRGPG)_2$ (C)
RP113	III_B	$(QRGPGR)_3$ (C)
RP114	III_B	R$(GPGR)_4$ (C)
RP116	III_B, MN	$(GPGRAF)_3$ (C)

Table 2. Fusion inhibition, neutralization, and ELISA titers of sera produced against peptides RP337, RP142, and RP116. Peptides RP337 and RP116 were conjugated to KLH before immunization whereas RP142 was not conjugated. Three guinea pigs were immunized, separately, with RP337, RP142, and RP116, and two rabbits were immunized with RP116. The sera from none of the three guinea pigs receiving RP116 were able to neutralize either the MN or III_B isolates. The immunization regimen was 100, 100, 50, and 50 μg of peptides for guinea pigs and 250, 250, 125, and 125 μg of peptides for rabbits. The fusion inhibition titer is the reciprocal of the dilution that reduces the number of syncytial foci by 90% (*2*). Neutralization titers are the reciprocal of the dilution that reduces reverse transcriptase activity by 50% (*2*). ELISA titers, done on plates coated with RP135, 142, and 116, are the serum dilution at which the immune serum is 0.21 optical density unit above the preimmune serum at 410 nm (*2*). Preimmune data are given for selected animals. Preimmune sera of other animals were negative in neutralization and ELISA. [Dashes (–), titers less than 10; ND, not done; g, guinea pig; R, rabbit.]

Sera	Fusion inhibition		Neutralization		ELISA		
	III_B	MN	III_B	MN	RP135 (III_B)	RP142 (MN)	RP116 (III_B)
g46, anti RP337	–	–	ND	ND	ND	ND	ND
g47, anti RP337	240	–	>2,560	16	25,000	8,000	24,000
g48, preimmune	–	–	–	–	<150	<150	<150
g48, anti RP337	20	40	800	>2,560	65,000	68,000	80,000
g88, anti RP142	–	10	20	480	<150	6,500	ND
g89, preimmune	–	–	–	–	<150	<150	<150
g89, anti RP142	80	80	1,400	>2,560	6,000	18,000	11,000
g90, anti RP142	–	20	–	640	<150	6,100	800
R374, preimmune	–	–	–	–	<150	<150	<150
R374, anti RP116	60	60	1,700	700	3,100	7,400	70,000
R375, preimmune	–	–	–	–	<150	<150	<150
R375, anti RP116	20	40	460	1,600	1,100	7,200	68,000

Table 3. Peptide blockade of sera using fusion inhibition assay. The concentration of each competing peptide was 50 μg/ml and the serum dilution was 1:10. CEM cells chronically infected with the indicated viral isolate were mixed with uninfected cells, serum, and peptide. The numbers of syncytia were determined in duplicate wells after 24 hours (*3, 6*).

Animal	Immunogen	Competing peptide	Number of syncytia per well	
			III_B	MN
g89	RP142 (MN)	None	0	0
		RP135	52	0
		RP142	54	39
		RP111	8	0
		RP113	0	0
		RP114	7	0
		RP116	60	0
g48	RP337 (III_B)	None	0	0
		RP135	42	38
		RP142	15	28
		RP111	7	0
		RP113	14	0
		RP114	12	0
		RP116	17	31
		Control (cells only)	60	44
R374	RP116	None	0	0
		RP116	70	66
R375	RP116	None	0	0
		RP116	65	55
		Control (cells only)	66	57

the animals immunized with RP337 neutralized and inhibited fusion of both MN and III_B. The ability to cross-neutralize was not dependent on the magnitude of the neutralization titer because serum from animal g47, immunized with RP337, neutralized III_B with a titer highter than that of animal g48 but did not neutralize the MN isolate.

A comparison of the amino acid sequence of RP337 and RP142 peptides suggests that antibodies binding to the peptide GPGRAF, common to both MN and III_B isolates, may be responsible for the cross-neutralizing activity. To test that, we used synthetic peptides in an attempt to block the fusion inhibition mediated by each of the two cross-neutralizing sera (Table 3). Serum from animal g89 (immunized with the MN PND, RP142) inhibited fusion of both the III_B and the MN isolates, and the immunizing peptide (RP142) abrogated the fusion inhibition activity against both III_B and MN. A similar 24–amino acid peptide, RP135, which contains the PND of the III_B isolate and in which the only common sequence with RP142 is GPGRAF, blocked fusion inhibition of the III_B isolate. The peptide RP116, consisting of a trimer of GPGRAF, blocked the III_B fusion-inhibiting antibodies, whereas peptides (RP111, RP113 and RP114) containing multimers of other short sequences did not. This confirms that GPGRAF is the sequence to which the cross-neutralizing antibodies bind. Neither RP135 nor RP116 abrogated the fusion inhibition of the homologous MN isolate, presumably because some of the antibodies in that sera bind sequences within the RP142 peptide not common to the PND of III_B and MN.

Similarly, RP142 and RP116 (both containing GPGRAF) were able to abrogate the MN fusion inhibition activity of the cross-neutralizing serum induced by RP337, the III_B PND peptide (animal g48). This shows again that the cross-neutralizing antibodies bind GPGRAF (Table 2). Sera produced by two rabbits immunized with RP116 (which contains only the GPGRAF sequence) inhibited cell fusion and neutralized both III_B- and MN-infected cells (Table 2). Peptide RP116 blocked fusion inhibition activities of the sera from these two rabbits (Table 3). This suggests that cross-neutralizing antibodies are elicited by immunization with only this common PND sequence.

To show that cross-isolate neutralizing sera recognize GPGRAF when this hexapeptide is flanked by several different sequences (as is found in different HIV-1 isolates), we performed enzyme-linked immunosorbent assays (ELISAs, Table 4) with

several 24–amino acid synthetic peptides containing the PND of independent HIV-1 isolates (*10*). Cross-neutralizing serum against RP337 recognized all peptides containing GPGRAF (RP142, 135, 145, 143, 150, and 141) and not those lacking this sequence (RP146, 144, 151, and 139). Absorption of this serum with GPGRAF-containing peptides RP150 and RP116 eliminated the reactivity to RP150, whereas absorption with RP139 (which lacks GPGRAF) did not (right side of Table 4). RP142 serum (g89) shows an ELISA pattern similar to that of g48 serum using this panel of peptides (*11*).

To show that antibodies binding GPGRAF neutralize randomly selected isolates that contain this sequence, we performed neutralization assays with four HIV-1 field isolates (Table 5). The sequence of the PND was determined for these isolates as described in (*10*), and two of these (6587-5 and 4489-5) contain GPGRAF in the PND. Both of these isolates are neutralized by the GPGRAF-specific sera (g48, g89, and both sera elicited by RP116), even though the sequences flanking the GPGRAF regions in these isolates are different. On the other hand, these sera have very low or undetectable neutralization titers against the isolates (7887-3, 6587-3, and RF) lacking the GPGRAF sequence in the PND. Overall, of seven isolates tested, four contain the GPGRAF sequence, and each is neutralized by antibodies binding this sequence.

We have recently analyzed the sequence of the PND from 245 HIV-1 isolates. The PND contains several sequences that occur in a large percentage of randomly selected virus isolates (*10*). One of the most prevalent is GPGRAF, which occurs in more than half of the 245 isolates. Of the 123 individuals analyzed (*10*), all the PND sequences from a majority of these individuals contained GPGRAF. The data presented here suggest that a large portion of HIV-1 field isolates will be neutralized by antibodies that recognize this sequence, thus making it an attractive candidate for vaccine efforts.

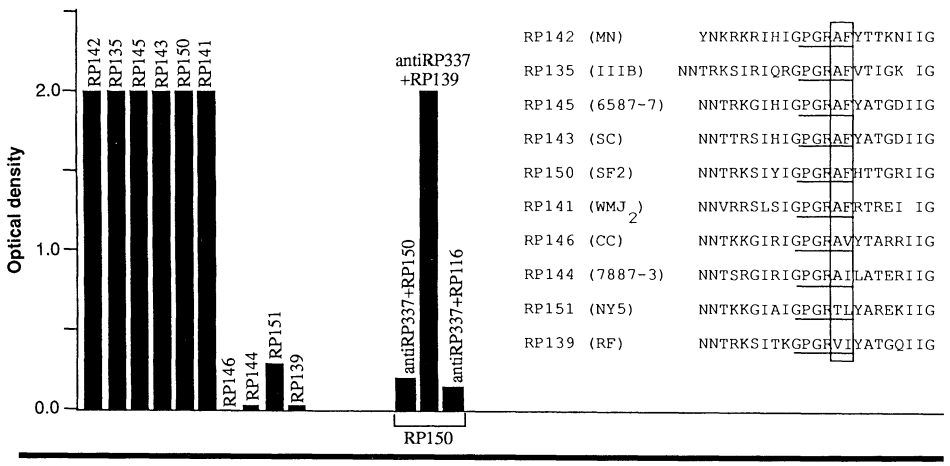
Our previous work has shown that PND-binding antibodies able to neutralize divergent isolates are not commonly elicited by a larger segment of the envelope or the PND (*1, 2, 12, 13*). This has led to the idea that the variable elements of the PND (non-GPGRAF sequences) are dominant for raising neutralizing antibodies. More studies should be done to develop approaches that will allow the conserved sequences of the PND to become consistently immunogenic, particularly in primate species. Nonetheless, our results indicate that the PND should not be considered solely a hypervariable domain because it also encompasses conserved regions that appear to be effective targets for antibodies that mediate cross-neutralization divergent isolates. At present we envision at least two specificities for neutralizing antibodies to the PND. (i) Type-specific neutralizing antibodies are highly isolate-restricted and responsive to the most variable portions of the PND. However, even in these variable segments there are a significant number of PND sequences similar to the consensus PND sequence (such as the MN isolate) (*10*), and antibodies recognizing these consensus-like sequences might be expected to neutralize more field isolates than, for example, those directed to the less common III_B/LAV-1 sequences. (ii) Group-common neutralizing antibodies are directed to more conserved regions of the PND, such as GPGRAF, and are able to neutralize otherwise divergent viruses such as MN and III_B.

It is possible that the ability of the virus to escape neutralization might be in part due to the absence of effective neutralizing antibodies to the PND conserved regions. Neutralization-resistant variants have been selected with PND-specific neutralizing antibodies, and sequence analysis of these mutants shows that the resistant phenotype is conferred by mutations both within and outside the PND (*14–19*). However, the antibodies used to generate these resistant variants were isolate-specific. It will therefore be of interest to determine whether the GPGRAF-binding antibodies described herein will neutralize such resistant mutants or escape variants that contain the GPGRAF sequence. These data provide some hope that vaccine prototypes able to elicit antibodies to conserved PND motifs may be effective in neutralizing a majority of HIV-1 isolates.

Table 4. ELISA results of g48 (RP337) antisera on a panel of 24–amino acid peptides analogous to RP135. The name of each isolate is shown in parentheses next to the peptide. The sequence of each peptide is shown. The serum was used at a 1:500 dilution. The ELISA plates were coated with the indicated peptides (1 μg/ml). The concentration of the competing peptide was 50 μg/ml.

(6587-5)	IHIGPGRAFH	940	2560	>2560	>2560
(7887-3)	IRIGPGRAIL	-	15	-	-
(6587-3)	LSIGPGRSFY	-	28	-	-
(4489-5)	IPIGPGRAFY	390	>2560	1500	800
(RF)	ITKGPGRVIY	-	-	-	-
(MN)	IHIGPGRAFY	>2560	>2560	700	1600
(IIIB)	IQRGPGRAFV	800	1400	1700	460

Table 5. Titers of antipeptide sera for neutralization of HIV-1 field isolates. A part of the sequence of the PND of each viral isolate used for the neutralization assays is shown (*10*). These isolates were obtained from patients infected with HIV. The neutralization titer is defined in Table 2.



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8 June 1990; accepted 26 September 1990

Secretion of Neurotoxins by Mononuclear Phagocytes Infected with HIV-1

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Mononuclear phagocytes (microglia, macrophages, and macrophage-like giant cells) are the principal cellular targets for human immunodeficiency virus-1 (HIV-1) in the central nervous system (CNS). Since HIV-1 does not directly infect neurons, the causes for CNS dysfunction in acquired immunodeficiency syndrome (AIDS) remain uncertain. HIV-1–infected human monocytoid cells, but not infected human lymphoid cells, released toxic agents that destroy chick and rat neurons in culture. These neurotoxins were small, heat-stable, protease-resistant molecules that act by way of *N*-methyl-D-aspartate receptors. Macrophages and microglia infected with HIV-1 may produce neurologic disease through chronic secretion of neurotoxic factors.

THE ACQUIRED IMMUNODEFICIENCY syndrome (AIDS) has a devastating effect on the central nervous system (CNS). Most clinical studies indicate that more than 60% of all patients with AIDS eventually develop cognitive dysfunction (*1*), and at least 25% show motor impairment (*2*). These disabilities range in severity from mental slowing and mild weakness to global dementia and paralysis. At autopsy, the CNSs of patients with AIDS show atrophy, white matter pallor, and modest loss of neurons (*1, 2*). Cellular changes within the brain include an invasion of macrophages, clusters of microglial cells (microglial nodules), and giant cell formation. The autopsy findings can, however, be rather sparse despite the fact that patients have had profound cognitive impairment (*3*).

The first isolations of human immunodeficiency virus-1 (HIV-1) from the brains of AIDS patients were reported by Shaw *et al.* (*4*) and Ho *et al.* (*5*). A number of other groups (*6*) have now identified mononuclear phagocytes (microglia, macrophages, and multinucleated macrophage-like cells) as the principal cellular targets for HIV-1 in the CNS. Since HIV-1 infection of neurons occurs very rarely, if at all (*1, 7*), the mechanisms impairing neuron function remain uncertain. Proposed etiologies for AIDS dementia have included the coexistence of opportunistic fungal or cytomegaloviral infections, white matter destruction by cytokines, growth factor blockade, and direct neuron killing by gp120, the HIV-1 envelope glycoprotein (*3, 8, 9*). We considered the possibility that HIV-1–infected mononuclear cells released substances that would disrupt the function of neurons (*3*). We tested for the presence of neurotoxins by means of two different culture systems. In the first, dissociated neurons from ciliary ganglia of embryonic chicks were seeded at low density on glass coverslips coated with poly-L-lysine. Under such conditions, neurons (about 50% of the total cell population) were readily identified by phase microscopy or histochemical markers (*10*). We also examined toxic effects on neurofilament (NF)–containing neurons from embryonic rat spinal cord (*11*) growing atop dense feeder layers of glia. [In these cultures <5% of the total population are NF^+ cells.] Several different types of $CD4^+$ human cell lines (the lymphoid cell H9, the promonocytic cell U937, and the monocytic cell THP-1) constitutively infected with $HTLV_{IIIB}$ prototypic strain of HIV-1 were used as models for HIV-1–infected human mononuclear cells (*12*).

Infected cells were seeded into chambers with filtered bottoms (0.4-μm pores) and then placed into culture wells containing neurons grown on cover slips (*13*). Using this coculture system to prevent direct contact between $CD4^+$ cells and neurons, we found that the HIV-1–infected monocytoid cells, U937 and THP-1, but not infected H9 lymphoid cells, secreted substances that reduced neuron growth and survival (Fig. 1). Dead or dying neurons could be detected within 10 hours of coculture, often showing

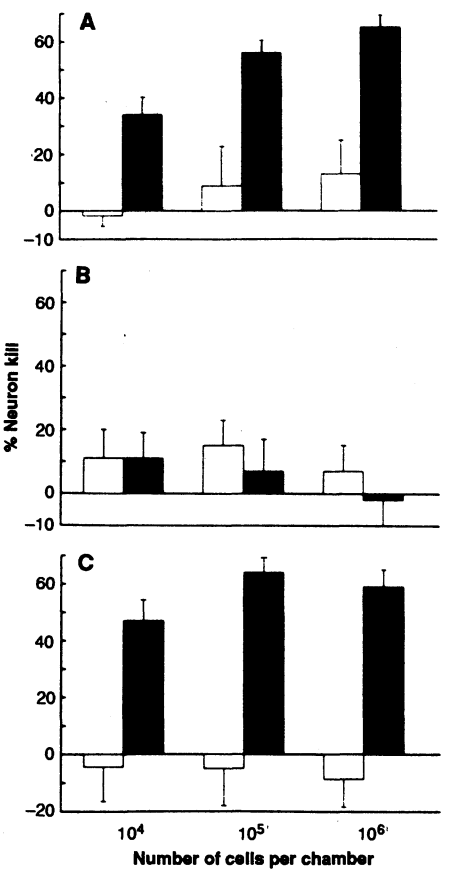

Fig. 1. HIV-1–infected monocytoid cells but not lymphoid cells release neuron-killing factors. The uninfected (white bars) or HIV-1–infected (black bars) cell lines were seeded at the indicated densities into Millicell-CM chambers which had been placed into wells (12-well plates; 3.8 cm^2 per well) with each well holding approximately 8000 chick ciliary neurons growing on a poly-L-lysine–coated glass cover slip in 1.0 ml of culture medium. The cocultures were incubated for 48 hours before fixation. (**A**) THP-1 monocytoid cells, (**B**) H9 lymphoid cells, (**C**) U937 promonocytoid cells. The percent neuron survival for each experiment was expressed as the ratio of the mean number of neurons per field for a treated culture to the mean number of neurons per field for untreated control cultures. Every value, expressed as mean percent neuron kill ± standard error (100% − % neuron survival), was obtained from at least three cultures. Negative percent kill indicates an increase in survival when compared to untreated control cultures. All comparisons between infected and uninfected THP-1 or U937 cells at each of the three cell concentrations showed $P < 0.05$ when analyzed by Student's *t* test.

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