interrelating the subicular complex, mammillary body, and anterior thalamus (*2*, *7*). A second hippocampal domain includes most of the CA1-subiculum field, which projects selectively and unilaterally to the enkephalin-GABA–rich LSr, which in turn projects to hypothalamic medial zone nuclei known to be involved in the expression of social behavior (agonistic and reproductive). And a third domain is restricted to the ventral tip of the CA1-subiculum field, which projects selectively and unilaterally to the GABA-estrogen receptor–rich (*12*) LSv, which in turn projects to the medial part of the medial preoptic nucleus and hypothalamic periventricular zone as a whole, which is known to preferentially influence ingestive behavior specifically as well as endocrine-autonomic responses that are associated with all types of behavior (*13*).

Evidence for subdivision of the second domain comes primarily from PHAL studies demonstrating that hypothalamic medial zone nuclei receiving LSr inputs project back to distinct though partly overlapping regions of the LSr (*9*, *14*). The topography of hippocampal inputs to the LS, and of bidirectional connections between the LS and hypothalamus described above, imply the following relation between the CA1-subiculum field and the hypothalamic medial zone. Via the LSr, ventral parts of the CA1-subiculum field (leaving aside the most ventral tip projecting to the LSv) are preferentially related to the medial preoptic nucleus (lateral part), which is involved in masculine sexual behavior (*15*); and progressively more dorsal parts of the CA1-subiculum field are related to the ventromedial-tuberal nuclei, which are involved in feminine sexual behavior (*16*); to the anterior hypothalamic nucleus, which is involved in agonistic behavior (*17*, *18*); and to the mammillary body (from the dorsal subiculum) (*7*).

The descending output of the hippocampal formation as a whole can be divided into two major components: the postcommissural fornix, which arises in the parahippocampal subicular complex and ends in the mammillary body and anterior thalamus; and the precommissural fornix, which arises in the hippocampus and ends in the LS and is divided here into three subcomponents (*3*, *7*). A functional distinction between the major post- and precommissural output channels is suggested by the identification of "navigation," "head direction," or "compass" neurons in the subicular complex and of "place" neurons in the hippocampus [see (*19*)]. Because all hippocampal pyramidal cells project through the precommissural fornix to the LS (*20*), it would appear that place neurons project topographically via the LS to hypothalamic systems thought to coordinate somatic (behavioral), endocrine, and autonomic responses associated with specific classes of motivated behavior. Cajal (*21*) pointed out that the LS is a medial part of the striatum (in the basal ganglia) innervated by hippocampal cortex, and it will now be important to determine the extent to which motor functions of the circuitry outlined here are similar in principle to those being clarified for the adjacent nucleus accumbens (ventral striatum) and caudoputamen (dorsal striatum).

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25. We thank G. Sanchez-Watts, D. M. Simmons, R. H. Thompson, G. D. Petrovich, E. A. Markakis, and A. E. Kelly for helping to prepare the in situ hybridization material; A. Tobin for the GAD65 probe; J. Habener for the SS probe; S. Sabol for the ENK probe; and R. B. Simerly for the use of certain PHAL material. Supported in part by NIH.

29 January 1996; accepted 3 April 1996

Infection and AIDS in Adult Macaques After Nontraumatic Oral Exposure to Cell-Free SIV

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Unprotected receptive anal intercourse is a well-recognized risk factor for infection with human immunodeficiency virus–type 1 (HIV-1). Isolated human case reports have implicated HIV-1 transmission by oral-genital exposure. Adult macaques exposed nontraumatically to cell-free simian immunodeficiency virus (SIV) through the oral route became infected and developed acquired immunodeficiency syndrome (AIDS). The minimal virus dose needed to achieve systemic infection after oral exposure was 6000 times lower than the minimal dose required to achieve systemic infection after rectal exposure. Thus, unprotected receptive oral intercourse, even in the absence of mucosal lesions, should be added to the list of risk behaviors for HIV-1 transmission.

Understanding the biology of HIV-1 is key to preventing its transmission. Epidemiologic studies have revealed that worldwide, most infections are acquired by mucosal exposure (*1*). In adults, known risk behaviors involving mucosal virus entry include vaginal and rectal intercourse. Oral infection is well documented in neonates, who can acquire the virus by breast feeding (*2*, *3*). Moreover, the presence of blood in gastric aspirates of neonates born to HIV-1–infected mothers was a risk factor for vertical transmission (*4*). These findings implicate the neonatal alimentary tract as a portal of virus entry during birth. Although some case reports have described seroconversion in adults after oral-genital sex only (*5*), a quantitative assessment of the relative risk of oral infection in adults has not been possible, as the route of virus entry is difficult to assess because of recall bias and problems inherent in face-to-face interviews.

Infection of rhesus monkeys (*Macaca mulatta*) with the SIV has proven to be the best system to model human HIV-1 transmission, viremia, and disease (*6*). HIV-1 and SIV are closely related: They share genome structure (*7*), modes of transmis-

sion, and target cells. Infected macaques develop clinical problems that are similar to those of HIV-1–infected humans, including $CD4^+$ T cell depletion, immunodeficiency, lymphomas, and central nervous system disease (6, 8). Thus far, oral infection of adult primates has not been shown. A single adult chimpanzee remained uninfected after oral exposure to a dose of HIV-1 that resulted in infection after intravaginal inoculation (9).

We previously reported development of a model for intrapartum virus infection through the mucosa (*10*). When cell-free SIV was administered orally to neonatal rhesus macaques at a dose of 300 50% animal infectious doses (AID_{50}) of SIV_{mac251} as measured by intravenous (iv) inoculation of adult macaques (AID_{50}-iv), all orally exposed neonates became infected and developed AIDS. These results led us to hypothesize that neonates may be very susceptible to infection after oral exposure, as a result of the decreased acidity of gastric secretions that is present at birth (*10*).

To test this hypothesis, two healthy, adult rhesus macaques were used in a pilot study and exposed to a well-characterized stock of SIVDeltaB670 (*11*), which had been titrated intravenously and intrarectally in adult macaques (Table 1). Animals M827 and N034 (Table 2) were pretreated with omeprazole, a potent inhibitor of gastric acid secretion (*12*), before oral exposure to either undiluted SIV or to a 1.2×10^{-3} dilution of virus (*13*). Both animals became infected. To test whether gastric acid neutralization was required for infection, the next animal (N530) was given the same virus dilution (1.2×10^{-3}) without omeprazole therapy. It also became infected systemically.

Next, we set out to determine the minimal infectious dose required to achieve nontraumatic oral infection of adult macaques without inhibiting their gastric acid secretion (Tables 1 and 2). A virus dilution of 8.3×10^{-6} resulted in infection (animal P329, Table 2), whereas a dilution of 4×10^{-6} did not (animal M730, Table 2). When the minimal dose of virus required to infect adult macaques orally was compared with that required for iv infection, we found that approximately 830 times more virus was needed to achieve oral infection (Tables 1 and 2). To confirm this result, we used the statistical method of Spouge (*14*) to determine the most likely titer of this virus stock for each route of infection. The AID_{50} for the oral route was approximately 5.7×10^{-6} [95% confidence interval (CI), 9.9×10^{-7} to 3.6×10^{-5}]. For the rectal route, the AID_{50} was 4.0×10^{-1} (95% CI, 9.1×10^{-2} to 2.1×10^{0}); this value is about 70,000 times greater than that for the oral route. The determination of the AID_{50}-iv, which yielded a titer of 2.2×10^{-7} (95% CI of 9.5×10^{-8} to 5.31×10^{-7}) according to the Spouge method (*14*), is problematic because a larger fraction of animals became infected at the dilution of 1×10^{-8} as compared with infection at dilutions that were 10 and 100 times lower. This "outlier" precludes the use of the Spouge method (*14*) to calculate the AID_{50}-iv, which assumes that all animals are equally susceptible to infection after exposure to a given dose of virus. In a genetically heterogeneous colony exposed to an uncloned virus preparation, this assumption may not hold. Consequently, we cannot determine whether the dose required to infect 50% of the adult macaques orally is significantly different from that required for iv infection.

Cell-free SIV stocks have been tested by other investigators for their ability to cross several mucosal barriers. Pauza *et al.* (*15*) found that approximately 100 to 1000 times more virus was required to achieve reproducible infection across intact rectal mucosa than was required for iv infection. Furthermore, in titrations of SIV by the intravaginal route, an inoculum 5000 to 10,000 times larger than that required for the iv route was needed to achieve infection of adult macaques (*16*). Systemic infection after conjunctival exposure has been reported also (*17*).

A dilution of 5×10^{-2} of cell-free SIVDeltaB670 yielded the minimal infectious dose required for nontraumatic infection through the intrarectal route; a dose that was 5×10^{6} times greater than that required for iv infection (Table 1). We were surprised to find that the minimal infectious virus dose needed to achieve nontraumatic mucosal infection after oral exposure was actually 6000 times lower than that needed for rectal infection.

In humans, intrarectal exposure is believed to carry a significantly higher risk of HIV-1 infection than do other mucosal routes. Thus far, unprotected oral-genital sex has been thought to carry a low risk of virus transmission (*18*). This expectation was not borne out in our macaque experiments, in which the minimal dose of cell-free SIV required for infection across the intact rectal mucosa exceeded that required for the oral route. Perhaps rectal infection in humans is facilitated by mucosal tears during sexual intercourse. Our controlled nontraumatic administration of cell-free virus to young adult macaques through rectal or oral exposure, however, demonstrated that the virus can cross intact upper gastrointestinal mucosal barriers more readily than it can cross intact rectal mucosa.

The orally infected adult macaques exhibited a pattern of viremia seen typically in iv-infected adults (Table 3). Acute peak viremia occurred within 2 weeks after inoculation, and chronic infection was seen in six of the seven orally exposed adult macaques. Within a maximal follow-up period of 214 days, two animals became moribund and were euthanized, whereas some survivors had more subtle signs of immune dysfunction. The two most recently inoculated animals continued to be asymptomatic (N539, P329; Tables 2 and 3). After exposing adult macaques orally to SIV, we did not observe transient infection without seroconversion, as was seen after rectal (*15*)

Table 1. Adult macaques were inoculated intravenously, intrarectally (by nontraumatic insertion of cell-free virus via an endoscope), or orally with serial dilutions of cell-free SIVDeltaB670 virus. Plasma and peripheral blood mononuclear cells (PBMCs), isolated from preservative-free heparinized blood, were fractionated on Ficoll-Hypaque density gradients and cocultivated with CEMx174 cells or human PBMCs as described (*10, 25*). Culture supernatants were tested for p27 antigen after 3 weeks of cocultivation. This assay does not cross-react with STLV-I or simian Type D retroviruses (*26*). Asterisks indicate one animal given undiluted virus and one of two animals given a dilution of 1.2×10^{-3} that were pretreated with omeprazole (5 milligrams per kilogram of body weight given intravenously every 8 hours, for a total of nine doses) (*12*) (see text) to inhibit gastric acid secretion. Virus was administered 4 hours after the last dose of omeprazole. This omeprazole regimen resulted in a gastric pH of 6.82 to 7.42 in a drug-control animal treated in parallel.

Virus dilution	Animals infected/ animals exposed (*n*)
Intrarectal	
5×10^{-1}	1/2
5×10^{-2}	1/4
5×10^{-3}	0/2
Oral	
3 ml undiluted	1/1*
1.2×10^{-3}	2/2*
1.3×10^{-4}	1/1
3.3×10^{-5}	1/1
8.3×10^{-6}	1/1
4.0×10^{-6}	0/1
Intravenous	
1×10^{-4}	2/2
1×10^{-5}	4/4
1×10^{-6}	3/4
1×10^{-7}	2/4
1×10^{-8}	2/2
1×10^{-9}	0/2
1×10^{-10}	0/2

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or intravaginal exposure (*16*), possibly because a small number of animals was used.

Our data do not imply that HIV-1 transmission by casual contact is likely to occur. Virus inocula representing 830 times the virus dose needed for the minimal iv infection are unlikely during casual contact, as has been borne out by several epidemiological studies. Specifically, kissing and the sharing of eating utensils or toothbrushes have not been associated with HIV-1 transmission (*19*). According to our results, the risk for oral infection appears to be limited to higher virus doses, as detected for example, in blood (*20*) or semen from viremic individuals (*1*, *21*). Indeed, the concentration of infectious virus particles (<1/ml) or infectious cells (<0.01%) is low in saliva, in contrast to blood (1 to 5000 infectious particles per milliliter; 0.001 to 1% infectious cells) or semen (10 to 50 infectious particles per milliliter; 0.01 to 5% infectious cells) (*1*). It should be noted also that protection against small virus inocula may be provided by inhibitory substances found in human saliva (*22*), which could render limited numbers of virions noninfectious.

An important issue for further study is the portal of virus entry after oral exposure. Because the susceptibility of adult macaques was considerably higher than expected and did not require gastric acid inhibition, we speculate that either gastric acidity has little influence on lentiviral infectivity or the portal of entry is proximal to the stomach. The recent establishment of human tonsillar histocultures provides an in vitro system in which to study the susceptibility of various cell types to HIV-1 (*23*). Possibly, dendritic cells located in tonsils, which have

Table 2. Adult macaques were exposed orally, without trauma, to dilutions of cell-free SIVDeltaB670 virus (Table 1). Omeprazole was given as outlined in the legend to Table 1. Whole blood samples with EDTA anticoagulant were evaluated for lymphocyte subsets by staining with fluorochrome-conjugated monoclonal antibodies and flow cytometry as described previously (*27*). Polymerase chain reaction (PCR) amplification was performed with nested primers specific for the SIV long-terminal repeat as described (*28*); the primers (*29*) were provided by B. Hahn (University of Alabama, Birmingham). Seroconversion was detected by protein immunoblots with the use of strips prepared commercially from HIV-2 antigens as described (*10*, *25*). m, male; f, female; pi, post-inoculation; CMV, cytomegalovirus; +, positive results; −, negative results.

Animal	Omeprazole	Virus dilution	PCR	Protein immunoblot	Clinical and pathological findings
M827 (f)	Yes	3 ml undiluted	+	+	Anemia; thrombocytopenia; transiently low $CD4^+CD29^+$ and $CD4^+$ T cell subsets; inverted $CD4^+/CD8^+$ ratios.
N034 (m)	Yes	1.2×10^{-3}	+	+	Anemia; low $CD4^+CD29^+$ T cell subset; inverted $CD4^+/CD8^+$ ratios; death due to cachexia on day 214 pi. CMV involvement of lungs and spinal cord; thymic atrophy; cryptosporidiosis of gall bladder and bile duct; syncytia typical of SIV in gut.
N530 (f)	No	1.2×10^{-3}	+	+	Anemia; thrombocytopenia; low $CD4^+CD29^+$ T cell subset.
N511 (f)	No	1.3×10^{-4}	+	−	Anemia; thrombocytopenia; low $CD4^+CD29^+$ T cell subset; death due to chronic wasting syndrome on day 105 pi. Colitis with crypt abscesses; meningitis; thymic atrophy; mesenteric lymphadenopathy; chronic focal cryptosporidiosis in gall bladder.
N539 (f)	No	3.3×10^{-5}	+	+	Asymptomatic by day 120 pi.
P329 (f)	No	8.3×10^{-6}	+	+	Asymptomatic by day 120 pi.
M730 (m)	No	4×10^{-6}	−	−	Healthy.

Table 3. Time course of viremia in adult macaques after oral exposure to SIVDeltaB670. Antigenemia, p27 levels in nanograms per milliliter; plasma cx, cocultivation of plasma with CEMx174 cells; results are listed as the 50% tissue culture infectious dose. PBMC cx, cocultivation of PBMCs with CEMx174 cells (the smallest number of PBMCs that yielded a positive culture is shown). Some samples were analyzed only for the presence (+) or absence (−) of virus. ND, not done. Plasma and PBMCs, isolated from preservative-free heparinized blood, were fractionated on Ficoll-Hypaque density gradients and cocultivated in duplicate cultures with CEMx174 cells as described (*10*, *25*). Culture supernatants were tested for p27 antigen after 3 weeks of cocultivation. Numbers in parentheses under "weeks after virus exposure" indicate weeks when corresponding tests were done.

Animal	Assay	Weeks after virus exposure										
		0	1	2	4	6	8	12 (14)	(15) 18	(22) 26	30	42 (45)
M827	Antigenemia	ND	2.72	0.98	0.00	0.00	0.00	0.04	0.22	0.05	0.07	(0.05)
	Plasma cx	ND	+	12	−	−	−	−	−	−		(−)
	PBMC cx	ND	+	1.56×10^6	+	+	+	9766	9766	9766		(+)
N034	Antigenemia	ND	0.00	7.30	0.78	0.19	0.40	0.50	0.27	0.49	0.39	
	Plasma cx	ND	+	215	−	−	−	−	−	6	−	
	PBMC cx	ND	+	2441	+	+	+	9766	9766	9766		
N530	Antigenemia	ND	0.00	0.99	0.00	0.00	0.00	(0.00)		(0.00)		0.12
	Plasma cx	−	−	−	−	−	−	(−)		−		+
	PBMC cx	−	−	+	+	−	1.56×10^5	(1.56×10^5)		(1×10^6)		+
N511	Antigenemia	ND	0.10	19.45	6.80	8.90	10.75	21.58	(32.57)			
	Plasma cx	−	−	+	>512			>512	(12,933)			
	PBMC cx	−	+	+	610			153	(153)			
N539	Antigenemia	0.00	0.00	0.00	0.00	0.00		(0.00)				
	Plasma cx	−	−	−	−	−		(−)				
	PBMC cx	−	−	+	+	−		(−)				
P329	Antigenemia	0.00	0.00	0.26	0.00	0.00		(0.00)				
	Plasma cx	−	−	6	−	−		(−)				
	PBMC cx	−	−	+	+	−		(−)				
M730	Antigenemia	ND	0.00	0.00	0.00	0.00	0.00	0.00	(0.00)		0.00	
	Plasma cx	−	−	−	−			−			−	
	PBMC cx	−	−	−	−			−			−	

been shown to support HIV-1 replication (*24*), are among the first virus targets in primates after oral exposure and play a key role in subsequent virus spread.

In sum, cell-free SIV is significantly more transmissible through the oral route as compared with the intrarectal route. The use of omeprazole, which results in the neutralization of gastric acid, was not required for infection. Because formal oral titrations were not conducted to establish the minimal infective dose in neonatal macaques (*10*), we do not know whether the oral exposure route is significantly more permissive of SIV entry in neonates as compared with adults. Our data, together with the case reports of HIV-1 seroconversion after oral-genital sex only (*5*), have implications for HIV-1 transmission. We conclude that oral exposure to infectious virus in the absence of mucosal lesions carries the risk of infection and AIDS. Thus, unprotected receptive oral intercourse should be added to the list of risk behaviors for HIV-1 transmission.

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30. All animal experiments were approved by the Animal Care and Use Committees at the Tulane Regional Primate Research Center and at the Dana-Farber Cancer Institute, which take responsibility for humane care and use of laboratory animals. We are committed to comply with the Principles for Use of Animals, the *Guide for the Care and Use of Laboratory Animals*, the Provisions of the Animal Welfare Act, and other applicable laws and regulations. The center's statement of assurance is on file with the USPHS, Office for Protection from Research Risks. These facilities are accredited by the American Association for Accreditation of Laboratory Animal Care. Animals are anesthetized with ketamine before all procedures that require the removal of animals from their cages. No restraining devices are necessary during these procedures. When necessary, moribund animals are euthanized by iv inoculation of a lethal dose of sodium pentobarbital.

31. We thank L. Corey for sharing unpublished data; A. Rosowsky for reviewing the manuscript; C. Lanclos, E. deHaro, E. Pecunia, and S. Kraus for technical assistance; and L. Geronimo for preparing the manuscript. Supported in part by NIH grants U01 AI24345, RO1-AI32330, RO1-AI34266, and RO1-AI35533 to R.M.R.; RR-00164, providing base support for the Tulane Regional Primate Research Center; AI-15093 and AI-35550 to M.M.-C.; and AI-15119 to L.N.M.; and by the Center for AIDS Research Core Grant no. IP30 28691 awarded to the Dana-Farber Cancer Institute as support for the iInstitute's AIDS research efforts. T.W.B. is a Scholar of the Pediatric AIDS Foundation and A.M.T. is a Fellow of the Minnie Burke Foundation.

15 February 1996; accepted 13 May 1996

Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals

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Certain chemicals in the environment are estrogenic. The low potencies of these compounds, when studied singly, suggest that they may have little effect on biological systems. The estrogenic potencies of combinations of such chemicals were screened in a simple yeast estrogen system (YES) containing human estrogen receptor (hER). Combinations of two weak environmental estrogens, such as dieldrin, endosulfan, or toxaphene, were 1000 times as potent in hER-mediated transactivation as any chemical alone. Hydroxylated polychlorinated biphenyls shown previously to synergistically alter sexual development in turtles also synergized in the YES. The synergistic interaction of chemical mixtures with the estrogen receptor may have profound environmental implications. These results may represent a previously uncharacterized level of regulation of estrogen-associated responses.

Reports of abnormal sexual development in reptiles (*1*, *2*) or birds (*3*) as well as feminized responses in male fish (*4–6*) have suggested an association with environmental chemicals functioning as estrogens. Similar hypotheses have been advanced in relation to an increased risk for breast cancer in women (*7*, *8*) and an observed decrease in human semen quality (*9*, *10*). A model for the developmental effects of estrogen is based, to a large extent, on studies with the synthetic estrogen diethylstilbestrol (DES) in animals and humans (*11–13*). Most estrogenic environmental compounds have potencies 1/50th to 1/10,000th those of DES or the natural estrogen 17β-estradiol; for example, the pesticides dieldrin, toxaphene, or endosulfan have an affinity for hER that is ~1/10,000th that of estradiol (*14*, *15*). The relatively low potencies of each of these compounds have suggested that these chemicals alone are unlikely to