# Protection Against Vaginal SIV Transmission with Microencapsulated Vaccine

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Although protection in animal models against intravenous challenges with simian immunodeficiency virus (SIV) has been reported, no previous vaccines have protected against a heterosexual route of infection. In this study, five of six macaques were protected against vaginal challenge when immunized with formalin-treated SIV in biodegradable microspheres by the intramuscular plus oral or plus intratracheal route. Oral immunization alone did not protect. After a second vaginal challenge, three of four intramuscularly primed and mucosally boosted macaques remained protected. The data suggest that protection against human immunodeficiency virus vaginal transmission could be provided by microsphere-based booster vaccines when used to immunize women who are systemically primed.

Human immunodeficiency virus (HIV) transmission through heterosexual intercourse in Africa and its rising incidence in North America and Europe (*1*) underscore the need to identify immunization strategies that will prevent infection across the genital mucosa. The higher incidence of heterosexual transmission to females indicates that any successful strategy will have to confer protection at the cervico-vaginal mucosa (*2*). To date, systemic intramuscular (IM) immunization with HIV or SIV recombinant proteins, HIV or SIV whole virus vaccines, and live-attenuated SIV have not been reported to protect against vaginal challenges but have been shown to protect against intravenous or rectal infection (*3–5*). The purpose of the present study was to establish a model for protection against vaginal transmission of HIV.

Antibodies in the cervico-vaginal mucus are present as the result of both passing from plasma and synthesis by local plasma cells (*6–8*). The plasma cell precursors that migrate to the uterus, cervix, and vagina are predominantly derived from mucosal rather than systemic lymphoid tissues and predominantly secrete immunoglobulin A (IgA) (*9*). Thus, the induction of these cells generally requires direct immunization of the mucosa-associated lymphoid tissues, such as those of the gastrointestinal or respiratory tracts (*10*), and is rarely achieved through parenteral immunization (*11*). Effective immunization of the mucosa-associated lymphoid tissues is difficult because of vaccine degradation and poor absorption at these surfaces. Numerous approaches to mucosal immunization are currently under investigation, including Ty particles, recombinant bacterial and viral vectors (*12*), and antigen-containing liposomes and microspheres (*13, 14*). We and others have demonstrated that antibody responses to vaccine antigens encapsulated in microspheres formulated from biodegradable polyester poly(DL-lactide-co-glycolide) (DL-PLG) are potentiated after systemic injection (*15*). In addition, when mucosally administered, the microspheres protect the vaccine from degradation, enhance absorption, and potentiate disseminated mucosal immune responses (*14, 16*).

The SIV model of acquired immunodeficiency syndrome (AIDS) in macaque monkeys has advantages for testing vaccination strategies designed to prevent the heterosexual transmission of HIV. The immune system of the female reproductive tract is similar to that of humans (*7, 17*), and infection can be readily transmitted by nontraumatic introduction of virus-containing fluids into the vagina (*18*). SIV spreads to the blood and lymphoid system in 1 to 2 weeks and AIDS develops over a period of 3 months to 2 years (*18*). We report that immunization of female rhesus macaques with microencapsulated SIV vaccine by various routes elicits both systemic and mucosal immunity and protects females against vaginal infection.

Gradient-purified SIVmac251 was treated with formalin before encapsulation with an emulsion-based process to produce 1- to 10-μm microspheres (*19*). Preliminary immunizations in BALB/c mice demonstrated that formalin treatment of the vaccine enhanced its stability and immunogenicity and that subcutaneous immunization with 50 μg of formalin-treated SIV in microspheres induced a 500-fold increase in plasma IgG specific for SIV as compared with soluble fixed vaccine (*20*).

Oral immunizations alone were first evaluated in a group of four female macaques. They received 5-ml suspensions of microencapsulated SIV in water through a gastrically inserted feeding catheter on experimental days 1, 52, 213, 643, and 670 (Fig. 1). The first three immunizations each contained 100 μg of vaccine protein, and the latter two contained 500 μg. Pre- and postimmunization samples of plasma and vaginal wash fluids were examined for the immunoglobulin class of antibodies to SIV

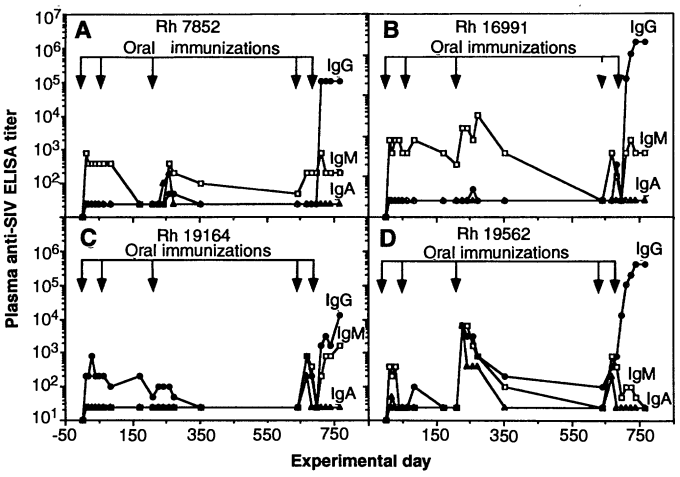


**Fig. 1.** Plasma SIV-specific antibody responses in female macaques immunized by the oral route with microencapsulated SIV vaccine and vaginally challenged with SIV (*26*). A 5-ml suspension of SIV vaccine (*19*) was administered into the stomach through a feeding catheter on experimental days 1, 52, 213, 643, and 670 (arrows). The first three immunizations each contained 100 μg of vaccine and the last two each contained 500 μg. Vaginal challenge with two vaginal $ID_{50}$'s of SIVmac251 was administered to all macaques on experimental day 684. IgM, IgG, and IgA titers of anti-SIV antibodies were determined in ELISAs (*21*) with the use of rhesus immunoglobulin class-specific antibodies (*22*).

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in enzyme-linked immunosorbent assays (ELISAs) (*21*) with disrupted SIV as capture antigen and goat antibodies to rhesus (anti-rhesus) IgM, IgG, and IgA as detecting antibodies (*22*). Each of the four macaques exhibited peak plasma antibody titers of 400 to 800 after the first oral immunization (Fig. 1). Most subsequent oral immunizations were followed by a rise in titer; however, the circulating antibody induced by wholly enteric immunization was 10- to 100-fold lower than that induced by IM immunization (Fig. 2A). The responses to first and subsequent oral immunizations were not uniform in immunoglobulin class distribution between individual macaques. IgM was highest in two animals (Fig. 1, A and B), IgG was highest in one (Fig. 1C), and all three classes were approximately the same in the fourth animal (Fig. 1D). Although the circulating IgA antibodies produced by three of four macaques after three enteric doses indicated that immunization of gut-associated lymphoid tissue had been achieved, SIV antibodies were not detected in vaginal wash fluids (*23*). All four orally immunized animals showed a large increase in IgG after vaginal challenge and were not protected (Table 1).

As a strategy for increasing mucosal antibody responses, microencapsulated SIV was evaluated as an oral and respiratory mucosal booster vaccination in macaques that had been primed intramuscularly. These protocols were based on previous results in macaques that demonstrated that IM immunization before oral or respiratory tract boosting primed for an accentuated secretory IgA response (*14*, *16*). Seven female macaques were administered three 100-μg IM injections into the thigh on experimental days 1, 52, and 213. All mounted circulating IgG titers from 102,400 to 1,638,400 and IgM titers from 200 to 6,400 (Fig. 2). Protein immunoblot analysis confirmed that each had responded to all major components of the virus, including the envelope glycoproteins (*23*). Beginning at 153 days after the last IM immunization, the primed macaques were administered two low- and two high-dose booster immunizations (Fig. 2). On experimental days 366, 402, 503, and 525 one macaque received booster immunizations by IM injection, three were boosted orally, and three were boosted by the intratracheal instillation (IT) of 1 ml of phosphate-buffered saline (PBS) in which encapsulated vaccine was suspended (*24*).

Intramuscular boosting induced high levels of SIV-specific plasma IgM and IgG (peak titers of 25,600 and 6,553,600, respectively), but no IgA response was detected (Fig. 2A). In each of the three macaques receiving oral boosters (Fig. 1, B to D), the decline in circulating SIV-specific IgG was halted, and each pair of oral doses resulted in two- to fourfold increases in this class of antibody. Plasma IgM was markedly increased and rose from minimally detectable levels to a titer of at least 3200. After the second pair of oral boosters, IgA was detected for the first time in three of three monkeys, but the increase was transient.

**Fig. 2.** Plasma anti-SIV responses in macaques immunized with microencapsulated SIV vaccine by IM injection and boosting via IM, oral, or IT routes. All macaques received 100-μg doses of SIV vaccine by IM injection on experimental days 1, 52, and 213. On experimental days 366, 402, 503, and 525 booster immunizations were administered by the IM (**A**), oral (**B** to **D**), or IT (**E** to **G**) routes. The first two boosters each contained 100 μg of vaccine and the latter two boosters each contained 500 μg of vaccine. A first vaginal challenge with two vaginal $ID_{50}$'s of viable SIVmac251 was administered to all macaques on experimental day 539. Titers of IgM, IgG, and IgA were determined in ELISAs (*21*).

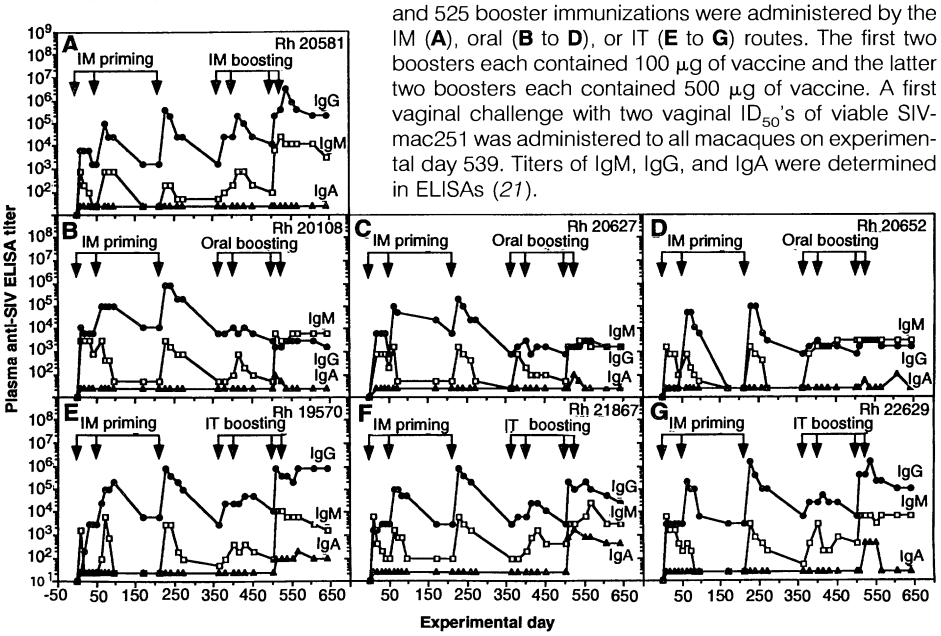
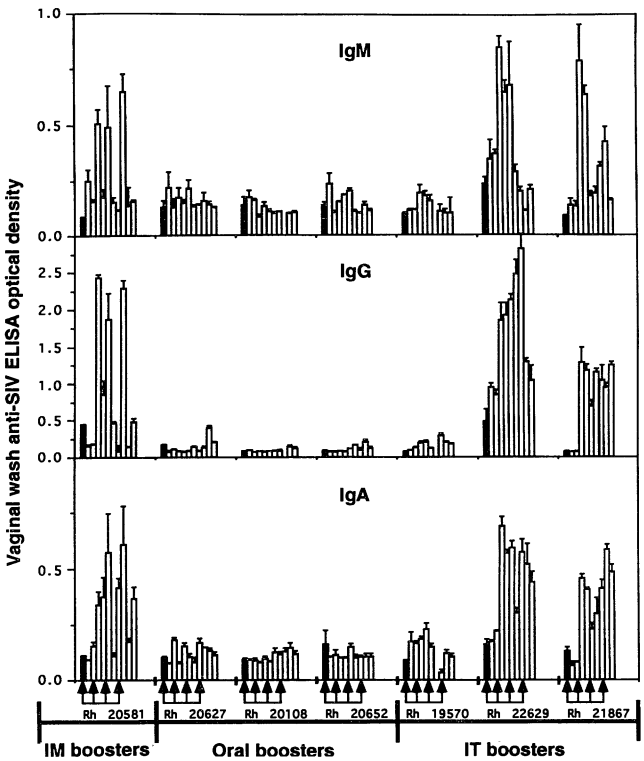



**Fig. 3.** Vaginal wash anti-SIV responses across the course of mucosal booster immunizations administered to systemically primed macaques. The optical density values of vaginal wash samples were obtained before the first day of mucosal boost (day 366, filled columns), and the effectiveness of the booster immunizations was judged relative to the premucosal boost values. The day of the experiment increases toward the right. Macaques that had been systemically primed on experimental days 1, 52, and 213 by IM injection of 100-μg doses of microencapsulated SIV vaccine were boosted on experimental days 366 (filled column), 402, 503, and 525 (four booster immunizations indicated by arrows) with microencapsulated SIV vaccine administered by the IM, oral, or IT route. A first vaginal challenge with two vaginal $ID_{50}$'s of viable SIVmac251 was administered to all macaques on experimental day 539. Vaginal wash fluids collected on experimental days 366, 379, 402, 414, 430, 454, 511, 525, 553, 567, and 609 were assayed for IgM, IgG, and IgA in ELISAs. Results are expressed as the mean ± SD optical density of 1:5 dilutions run in triplicate, and the scales range from 0.0 to 1.0 for IgM and IgA and 0.0 to 3.0 for IgG.



Boosting by the IT route was more effective because 64- to 512-fold rises in plasma IgG and IgM (Fig. 2, E to G) were seen. The second pair of high-dose IT boosters induced levels of IgG and IgM comparable to those induced by IM boosting. In addition, substantial circulating IgA antibodies to SIV (anti-SIV) were made by all three macaques boosted by the IT route, and in two of three macaques the IgA response remained elevated for more than 130 days.

Samples of vaginal fluids were collected from the seven systemically primed macaques across the period when mucosal boosters were administered. Class-specific ELISAs for anti-SIV demonstrated that at a 1:5 dilution, the vaginal washings from all seven macaques contained specific antibody activity relative to samples taken immediately before mucosal boosting (Fig. 3). Some of the vaginal wash fluids from the single macaque receiving IM boosters contained high levels of IgM, IgG, and IgA. However, the level of antibodies tended to be high only in those samples collected immediately after the IM boosters. In contrast, two of three macaques boosted by the IT route exhibited vaginal wash IgG and IgA levels that were high in all samples collected after the second mucosal booster immunizations and the third had a modest increase (Fig. 3). The oral booster immunizations proved much less effective in the induction of antibody secretion into vaginal fluids, although three of three macaques exhibited IgG, and two of three exhibited IgA, anti-SIV levels that were significantly above premucosal boosting levels. Thus, IgG anti-SIV was detectable in vaginal wash fluids of all seven macaques after the last booster immunization. In addition, IgG was the predominant component of the response and end point titration showed that it was generally present at 16 to 32 times the level of either specific IgM or IgA (*22*).

The degree of protection provided at the cervico-vaginal mucosa by the various mucosal immunization regimes was tested by vaginal application of a live homologous SIVmac251 stock that had been previously titrated for both intravenous and vaginal challenges (*18*). Fourteen days after the last immunization, the four macaques from the oral only protocol (Fig. 1), the six macaques from the systemic priming plus mucosal boosting protocol (Fig. 2), the one IM only immunized macaque, and four nonimmunized control macaques were challenged by the nontraumatic application of two vaginal median infective doses ($ID_{50}$'s) (approximately 10,000 IV animal infectious doses) to the vaginal epithelium. At 2, 4, 6, and 8 weeks after challenge, blood mononuclear cells were cocultured with CEM×174 cells to detect SIV infection (*5*, *18*). Table 1 shows that three of four nonimmunized control macaques, four of four macaques from the oral only protocol, but only one of six macaques from the systemic-mucosal protocols were SIV-positive (Rh 19570, IM-IT was positive) at all time points. One additional macaque from the systemic-mucosal protocol (Rh 22629; IM-IT) tested weakly positive at week 4 but has been negative at all later time points for up to 1 year after challenge.

Fifteen weeks after the first vaginal challenge, the one IM-IM macaque, all three IM-oral macaques, and the one IM-IT–immunized macaque that had shown no indication of infection were boosted with microencapsulated SIV vaccine by the same route as their previous mucosal boosts. Fourteen days later, together with the single remaining uninfected control monkey, they were re-challenged by the vaginal application of two vaginal $ID_{50}$'s of SIVmac251. Sequential peripheral blood–CEM ×174 cell cocultures showed that the final nonimmunized control (Rh 60P), the IM-IM (Rh 20581), and one of the IM-oral–immunized macaques (Rh 20108) became infected (Table 1). Therefore, three of four macaques receiving IM priming followed by mucosal boosting resisted two vaginal challenges 15 weeks apart (two vaginal $ID_{50}$'s each), whereas four of four naïve controls and five of five receiving only IM or only oral immunizations became infected.

These data show protection against live SIV challenge at the cervico-vaginal mucosa and suggest that this protection was dependent on mucosal immunization with a microsphere-based vaccine delivery system. However, the high but apparently short-lived amounts of antibodies in the vaginal wash of the macaque receiving IM boosters (Fig. 3) raises the question of whether or not the choice of the thigh as the site of systemic immunization may have fortuitously primed for a genital antibody response after mucosal boosting. The iliac lymph nodes have been reported to supply antibodies to the female reproductive tract (*8*), and IM immunization into the thigh may have stimulated these tissues.

**Table 1.** Isolation of SIV from blood mononuclear cells of vaginally challenged macaques. Fourteen days after the last immunization with microencapsulated SIV vaccine (Figs. 1 and 2), all macaques were challenged by nontraumatic application of two vaginal $ID_{50}$'s of SIVmac251 (1.0 ml of undiluted stock) as previously described (*18*). Mononuclear cells were isolated from heparinized blood by centrifugation over Ficoll-Hypaque. Mononuclear cells ($2 \times 10^7$ to $3 \times 10^7$) were cocultured with $5 \times 10^6$ CEM×174 indicator cells. Culture supernatants were tested weekly for reverse transcriptase and p27 antigen as described (*18*). Positive cultures displayed cytopathic effects typical of SIV, reverse transcriptase, or p27 antigen from 1 to 4 weeks after initiation of culture. Negative cultures were tested weekly for 8 weeks before being discarded. At 15 weeks after the first challenge, the virus-negative animals were boosted with microencapsulated SIV vaccine by the same route as their previous immunization and challenged 14 days later with two vaginal $ID_{50}$'s. Infection of the control macaques was also confirmed by seroconversion to SIV and recombinant gp140 (*23*). All animals were cultured for virus for up to 1 year after the first vaginal challenge. All protected animals were virus culture–negative even when $30 \times 10^6$ peripheral blood mononuclear cells were cocultivated with CEM×174 cells. Likewise, unprotected animals were repeatedly virus-positive in the peripheral blood. Polymerase chain reaction amplification of DNA from peripheral blood cells was negative for SIV DNA for all animals still protected after the second challenge and was positive for SIV DNA from an infected animal included as a control (*23*).

| Immunization | Macaque number | SIV isolation from blood mononuclear cells | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Weeks after first challenge | | | | Weeks after second challenge | | | |
| | | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 |
| Negative control | 60P | − | − | − | − | + | + | + | + |
| | 127L | + | + | + | + | | | | |
| | 240M | + | + | + | + | | | | |
| | 249N | + | + | + | + | | | | |
| Oral-oral | 7852 | + | + | + | + | | | | |
| | 16991 | + | + | + | + | | | | |
| | 19164 | + | + | + | + | | | | |
| | 19562 | + | + | + | + | | | | |
| IM-IM | 20581 | − | − | − | − | + | + | + | + |
| IM-oral | 20108 | − | − | − | − | + | + | + | + |
| | 20627 | − | − | − | − | − | − | − | − |
| | 20652 | − | − | − | − | − | − | − | − |
| IM-IT | 19570 | + | + | + | + | | | | |
| | 21867 | − | − | − | − | − | − | − | − |
| | 22629 | − | + | − | − | | | | |

Although SIV-specific antibodies were demonstrable in the vaginal wash fluids of all protected macaques before both the first and second challenges, their precise role in protection has not been established. Several recent publications have implicated antibodies directed to antigens common to the cell line (or lines) used for the in vitro propagation of the virus for vaccine and challenge stocks (*25*). To address this point, we examined samples of plasma and vaginal wash fluids from the macaques for the presence of antibodies to the HuT-78 cell line that was used to produce the SIV for microencapsulation. As determined by flow cytometric analysis, only plasma from the single macaque that received only IM immunizations (Fig. 2A) and that was not protected (Table 1) had detectable levels of antibodies to HUT-78 cells (*23*). In contrast, all macaques immunized with mucosal boosting were negative in immunofluorescence flow cytometric assays for plasma antibodies against HuT-78 cells, but the plasma from five of six macaques specifically stained HuT-78 cells that were infected with a *vaccinia* virus vector carrying the gene for SIVmac251 gp140 (*23*). The data suggest that antibodies against cell antigens did not play a critical role in the observed protection. In addition, the finding that most of the macaques with low levels of vaginal wash SIV antibodies resisted challenge suggests that partial protection against vaginal transmission may be achieved with relatively low levels of mucosal antibodies provided that the antibodies are consistently present. However, the possible involvement of cell-mediated immunity in the protection has not been addressed.

Although the precise mechanism of protection needs to be established by further investigation and both the role and amount of vaginal antibody needs to be addressed, the results provide evidence in a relevant animal model that protection against vaginal transmission of HIV is possible through vaccination. Improved approaches to mucosal boosting with microencapsulated vaccines may be important in the protection of mucosal surfaces against HIV.

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19. SIV was formalin-treated at a final concentration of 0.0925% at 22°C for 18 hours, centrifuged to remove excess formalin, and microencapsulated in DL-PLG by an emulsion-based process. A 5-mg pellet of virus was resuspended in 100 μl of deionized water ($dH_2O$) before mixing with 0.5 g of 54:46 DL-PLG (Southern Research Technologies, Birmingham, AL) that had been dissolved in 4 g of reagent-grade methylene chloride (Eastman Kodak, Rochester, NY). The polymer solution was then added, with mixing, to 60 ml of an 8% (by weight) aqueous solution of poly(vinyl alcohol) (Air Products and Chemicals, Allentown, PA) to produce an oil-in-water emulsion. The microspheres were then hardened by addition of the emulsion to 3.5 liters of $dH_2O$ with stirring. The resulting microspheres were collected by centrifugation, washed twice in $dH_2O$ to remove residual poly(vinyl alcohol), and collected by lyophilization. The sizes of the preparations were determined by particle size analyzer (Malvern Instruments, Malvern, United Kingdom). Ninety-five percent of microspheres had a Poisson distribution of 1 to 10 μm in diameter.
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21. ELISAs were done in plates coated with 100 μl per well of a titrated solution of freeze-fractured SIV in borate-buffered saline (BBS) overnight at 2°C. The plates were blocked for 2 hours at room temperature with 1% bovine serum albumin in BBS, which was also used as the diluent. All washes were with PBS containing 0.05% Tween-20. One hundred microliters of various twofold dilutions of specimens were added to washed triplicate wells and incubated for 6 hours at room temperature. After washing, 100 μl of diluted biotinylated anti-rhesus IgM, IgG, or IgA antibody were added to each well and incubated overnight at 4°C. After the removal of the unbound biotin conjugate by washing, 100 μl of horseradish peroxidase–conjugated avidin was added, and the plates were incubated for 2 hours at room temperature. Color was developed with the substrate 2-2′-azino (3-ethylbenz thiozoline sulfonic acid) (0.3 mg/ml) in citrate buffer (pH 4.0) containing 0.0003% $H_2O_2$ and read at 415 nm on a Kinetics Reader, model EL 312 (Biotek Instruments, Winooski, VT). The results are presented as the reciprocal of the greatest sample dilution producing a signal significantly greater than that of the animal-matched preimmunization sample at the same dilution (end point titration).
22. Immunoglobulins were purified from rhesus serum by precipitation at 50% saturation with ammonium sulfate followed by anion-exchange chromatography on DEAE-cellulose (DE-52; Whatman, Kent, England). The fractions containing each of the immunoglobulin classes were identified by precipitation in gel with selected lots of affinity-purified goat antibodies to human (anti-human) IgM, IgG, and IgA (Southern Biotechnology Associates, Birmingham, AL) and separately pooled. Sizing chromatography of the IgG fraction on Ultrogel AcA 34 (IBF Biotechnics, Columbia, MD) in BBS yielded pure IgG. The IgM fraction was purified by sequential chromatography on AcA 34 and AcA 22. In the case of the serum IgA, it was necessary to isolate the relatively minor dimer fraction by means of AcA 34 to remove all contaminating IgG. Secretory IgA was purified from rhesus colostrum that had been acidified to pH 2.5 with HCl to precipitate the bulk of the nonimmunoglobulin proteins followed by precipitation at 50% saturation with ammonium sulfate. Pure secretory IgA was obtained by sequential chromatography on AcA 22 and AcA 34. The isolated rhesus serum immunoglobulins were subjected to immunoelectrophoresis in thick agarose gels and were precipitated with the corresponding goat anti-human IgM, IgG, or IgA. The precipitin lines were cut from the gels, emulsified in complete Freund's adjuvant, and used to immunize goats. Affinity isolations of the anti-rhesus IgM, IgG, and IgA were done on columns of solid-phase–bound human myeloma proteins of the corresponding isotype. Final cross-adsorptions to specificity used the appropriate combination of columns with solid-phase–bound rhesus serum IgM, serum IgG, and colostral IgA. Characterization of these reagents by ELISA demonstrated equivalent sensitivity in the detection of their respective classes of immunoglobulins.
23. P. A. Marx, R. W. Compans, J. H. Eldridge, unpublished results.
24. Fasted monkeys were anesthetized by IM injection of ketamine hydrochloride. Gastric immunization was through an 8 French by 40-inch pediatric feeding tube (Becton Dickinson) inserted into the stomach. Intratracheal intubation was accomplished with an 8 French by 15-cm pediatric tracheal tube that had been inserted through the glottis with the use of a 6 French intubating stylet. After the tracheal tube had been taped in place, the animal was briefly ventilated with humidified 100% oxygen to prevent hypoxemia. The 1-ml suspension of vaccine-containing microspheres in PBS was instilled with the use of a syringe through an 8 French by 15-inch catheter that had been inserted 1 cm past the end of the tracheal tube. An additional 1 ml of PBS was then passed through the catheter to clear the dead volume.
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26. Protocols for animals used in this study were reviewed and approved by institutional animal care and use committees. When anesthesia was required by the protocol (average of once per month or less), animals were lightly anesthetized with Ketamine HCl at 10 mg per kilogram of body mass.

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# High Spontaneous Intrachromosomal Recombination Rates in Ataxia-Telangiectasia

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**Ataxia-telangiectasia (A-T) is an inherited human disease associated with neurologic degeneration, immune dysfunction, and high cancer risk. It has been proposed that the underlying abnormality in A-T is a defect in genetic recombination that interferes with immune gene rearrangements and the repair of DNA damage. Recombination was studied in A-T and control human fibroblast lines by means of two recombination vectors. Unexpectedly, spontaneous intrachromosomal recombination rates were 30 to 200 times higher in A-T fibroblast lines than in normal cells, whereas extrachromosomal recombination frequencies were near normal. Increased recombination is thus a component of genetic instability in A-T and may contribute to the cancer risk seen in A-T patients.**

Ataxia-telangiectasia is an autosomal recessive disease characterized by progressive cerebellar ataxia, immune defects, progeric changes of the skin, endocrine disorders, gonadal abnormalities, and a high incidence of cancer (*1*, *2*). Heterozygote carriers may also be at increased risk for cancer, particularly carcinoma of the breast (*2*). Patients with A-T are classified into four complementation groups (A, C, D, and E) on the basis of heterodikaryon complementation studies; however, there are no consistent phenotypic differences between complementation groups (*3*). The underlying defect in A-T is unknown, but A-T cells exhibit abnormalities consistent with a defect that involves DNA metabolism or the maintenance of genomic integrity: for example, frequent chromosome aberrations, sensitivity to the killing effects of ionizing radiation, and radiation-resistant DNA synthesis (*3*). Other A-T abnormalities may be the consequence of aberrant immune gene rearrangements. During normal development, the immunoglobulin (Ig) heavy chain gene and T cell receptor (TCR) genes undergo rearrangements that result in the production of IgA, IgE, IgG2, and IgG4 and the expression of α/β chain TCRs (*4*). Patients with A-T have deficiencies in these Ig classes (*1*), a relative lack of T cells expressing α/β chain TCRs (*5*), elevated frequencies of aberrant interlocus TCR gene rearrangements (*6*), and a high frequency of lymphocytes that contain chromosome translocations involving sites near immune genes [reviewed in (*7*)]. Several investigators have suggested that a defect in genetic recombination resulting in an inability to productively rearrange and repair genes would provide a unifying explanation for these immune defects and would also account for the karyotypic abnormalities and radiation sensitivity in A-T (*5*, *7*, *8*). We tested this hypothesis by measuring spontaneous mitotic recombination between directly repeated genes contained in vectors that had been integrated into the chromosomal DNA of A-T, xeroderma pigmentosum (XP), and control human fibroblasts.

Recombination first was measured by means of the retroviral vector pNeoA (Fig. 1A). This vector contains three antibiotic resistance genes driven by independent promoters: a hygromycin resistance gene (*hph*), used to select for stable transformants, surrounded by two mutant genes encoding neomycin resistance (*neo*) that are each defective because of different 8–base pair (bp) linker insertions. A 299-bp deletion of transcriptional enhancer elements in the 3′ long terminal repeat (LTR) of the vector is transferred to the 5′ LTR during infection, rendering the integrated pNeoA provirus transcriptionally silent. As a result, there is no vector mRNA production in infected human cells that might confound interpretation of recombination data. Gene conversions, unequal sister chromatid exchanges (SCEs), and reciprocal intrachromatid exchanges between the *neo* genes can be detected when they result in the reconstitution of a wild-type gene and expression of G418 resistance (Fig. 1B).

After pNeoA was transfected into the mouse retroviral packaging cell line PA317,

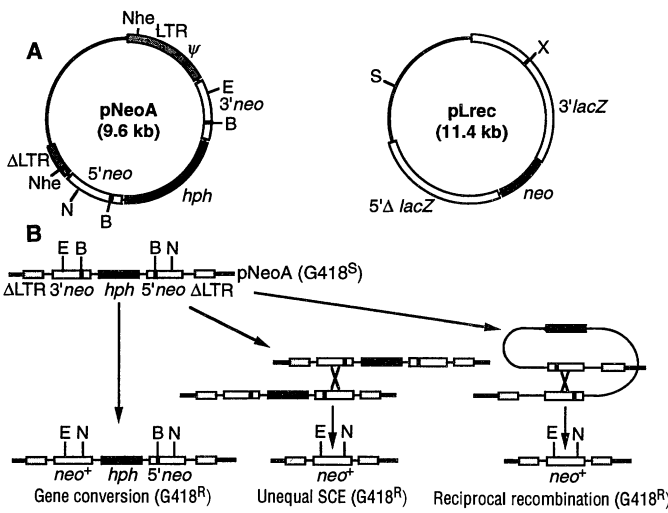


**Fig. 1.** The pNeoA and pLrec recombination vectors. (**A**) Vector maps. The pNeoA was constructed as described (*9*) by the insertion of two copies of *neo* from pCMIneo-(polA) along with the *hyg* from pHyg into the polylinker region of the retroviral vector pB2d (*27*). The Nae I site of the left-hand *neo* and the Eag I site of the right-hand *neo* have been destroyed by the insertion of 8-bp Bam HI linkers. The pLrec was constructed as described (*15*) by the insertion of an 8-bp Xho I linker into the Hpa I site 1351 bp from the start of the *lacZ* of pCH110 (*28*) and then the insertion of *neo* from pCMIneo(polA) and a 3-kb Hpa I–Bam HI *lacZ* fragment of pCH110 into the Bam HI site of the modified pCH110 plasmid. Restriction sites: B, Bam HI; E, Eag I; N, Nae I; Nhe, Nhe I; S, Sca I; and X, Xho I. Abbreviations: ψ, retroviral packaging signal sequence; ΔLTR, LTR containing a 299-bp deletion; and G418S, G418 sensitivity. (**B**) Intrachromosomal recombination of the integrated pNeoA vector. When integrated into genomic DNA, the pNeoA vector can undergo several different recombinational events that lead to reconstitution of wild-type *neo*, which confers G418 resistance (G418R): nonreciprocal transfer of information from one *neo* to another (gene conversion) and reciprocal exchanges that involve loss of the duplication and deletion of sequence between the points of exchange (unequal SCE and reciprocal intrachromatid exchange). The integrated pLrec vector can undergo the same rearrangements, leading to reconstitution of a wild-type *lacZ*.

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