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Journal Title: Journal of Virology
Volume: Volume 85, Number 9
Publisher: American Society for Microbiology | 2011-05, Pages 4547-4557
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JVI.02411-10
Permanent URL: http://pid.emory.edu/ark:/25593/f15vg

Final published version: http://jvi.asm.org/content/85/9/4547

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Accessed September 5, 2019 3:10 AM EDT
Infection, Viral Dissemination, and Antibody Responses of Rhesus Macaques Exposed to the Human Gammaretrovirus XMRV

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Received 18 November 2010/Accepted 7 February 2011

Xenotropic murine leukemia-related virus (XMRV) was identified in association with human prostate cancer and chronic fatigue syndrome. To examine the infection potential, kinetics, and tissue distribution of XMRV in an animal model, we inoculated five macaques with XMRV intravenously. XMRV established a persistent, chronic disseminated infection, with low transient viremia and provirus in blood lymphocytes during acute infection. Although undetectable in blood after about a month, XMRV viremia was reactivated at 9 months, confirming the chronicity of the infection. Furthermore, XMRV Gag was detected in tissues throughout, with wide dissemination throughout the period of monitoring. Surprisingly, XMRV infection showed organ-specific cell tropism, infecting CD4 T cells in lymphoid organs including the gastrointestinal lamina propria, alveolar macrophages in lung, and epithelial/interstitial cells in other organs, including the reproductive tract. Of note, in spite of the intravenous inoculation, extensive XMRV replication was noted in prostate during acute but not chronic infection even though infected cells were still detectable by fluorescence in situ hybridization (FISH) in prostate at 5 and 9 months postinfection. Marked lymphocyte activation occurred immediately postinfection, but antigen-specific cellular responses were undetectable. Antibody responses were elicited and boosted upon reexposure, but titers decreased rapidly, suggesting low antigen stimulation over time. Our findings establish a nonhuman primate model to study XMRV replication/dissemination, transmission, pathogenesis, immune responses, and potential future therapies.

Xenotropic murine leukemia-related virus (XMRV) is a novel gammaretrovirus, initially identified in human prostate cancer using a Virochip DNA microarray (43) in men with a low-activity variant of RNASEL, an enzyme involved in innate immunity via type I interferons (14). Although related to murine leukemia virus (MLV) and probably acquired by zoonotic infection, human tissue-derived XMRV clearly segregates from other gammaretroviruses, genotypically arguing against the hypothesis that such human infection is acquired via repeated zoonotic transmission (43). The association of XMRV with prostate cancer has since been confirmed by other laboratories, albeit with a potentially different cellular tropism (34). In addition, association with RNASEL deficiency has been variable (1, 7, 15, 34), suggesting that low levels of RNASEL may not be a requirement for productive infection or viral propagation in humans. Nevertheless, the association of RNASEL mutations and prostate cancer has been reinforced by the recent discovery that a prostate cell line, 22Rv1, was derived from a patient with a low-activity RNASEL genotype (15). RNASEL dysfunction has also been associated with another disease, chronic fatigue syndrome (CFS) (8, 20, 21, 38, 42), which prompted an investigation into a potential association of XMRV with CFS. In a geographically restricted cohort, up to 67% of CFS patients were found to harbor XMRV in their blood (17), which led to a number of similar studies in the United States and Europe. Thus far, data from all European cohorts failed to demonstrate a similar association (6, 9, 44), suggesting that the association may be either restricted to the United States and/or the result of different testing methods (19). Numerous host restriction factors, including cytidine deaminases of the APOBEC family and tetherin, are important in determining XMRV infectivity (2, 10, 27). Although presumably derived from an endogenous murine virus, laboratory mice have a polymorphism of its putative receptor, XPR1, preventing infections (45) except for a few exceptions (16, 32). Because of its association with two important human diseases, prostate cancer and CFS, the establishment of a suitable animal model is critical to determine if XMRV is infectious and, if so, to understand its replication kinetics, dissemination in vivo, and the nature of the host response elicited by XMRV. For these reasons, we chose the Indian rhesus macaque, a nonhuman primate that is evolutionarily close to humans, as an animal model for XMRV infection.

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© Published ahead of print on 16 February 2011.
MATERIALS AND METHODS

Animals. A total of eight adult healthy rhesus macaques (five infected and three negative controls) of Indian origin were used for this study, with ages ranging from 5 to 17 years. The nonhuman primates were housed at Yerkes National Primate Research Center at Emory University and maintained in accordance with the instructions of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, and the U.S. Public Health Service (PHS) Guidelines (24).

Blood and tissue sampling. Blood was collected by venipuncture, and bone marrow from the iliac crest and peripheral lymph nodes were surgically removed at days 7 and 14 postinfection (p.i.). At necropsy, a complete collection of organs was sampled.

XMRV inoculations. Monkeys were inoculated with 5.6 × 10^6 90% tissue culture infective doses (TCID50) of XMRV grown in DU145 prostate cancer cells delivered intravenously as outlined in Fig. 1a. Given the apparent disappearance of the virus from blood, two monkeys were reinfected at day 158 postinfection with 3.6 × 10^6 TCID50 of sucrose-purified XMRV (Advanced Biotechnologies, Inc., Columbia, MD) delivered intravenously (i.v.). Euthanasia was performed on day 4548 ONLAMOON ET AL. J. VIROL.

Phenotypic characterization by flow cytometry. Peripheral blood mononuclear cells (PBMC) were stained with fluorochrome-labeled antibodies to CD14, -CD20, -CD3, and -CD8 markers. The presence of XMRV proviral DNA was amplified by nested PCR using QIAamp Blood DNA Mini Kit following the manufacturer's instructions (Qiagen). Each run was performed with adequate negative and positive controls as described elsewhere (11).

Nested PCR. DNA from total PBMC and fractionated blood samples was extracted using QIAamp Blood DNA Mini Kit following the manufacturer's protocol. The presence of XMRV proviral DNA was amplified by nested PCR using oligonucleotides 5922F and 6273R for round 1 and 5942F and 6200R for round 2 (11), designed for the env region. For first-round PCR, 200 to 250 ng of DNA was used. Twenty-five microliters of FideliTag Hotstart IT Mastermix (USB Corp.) was used in a total reaction volume of 50 μl supplemented with 2 μl of 25 mM MgCl2 in a total volume of 50 μl. The PCR conditions were 94°C for 4 min for the initial denaturation, followed by 45 cycles consisting of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s, with a final extension of 5 min at 72°C. Three microliters of the first-round reaction product was used for the second round using the same buffer composition and conditions with the exception that the program consisted of 35 cycles. The PCR products were purified using a Wizard SV PCR and gel cleanup system (Promega, WI), and, aligned with known XMRV sequences. To test for the presence of full-length XMRV DNA, 419F (5'-ATC AGT TAA CCT ACC CGA GTC GGA C-3') and 8165R (5'-TTG CAA AGA GCA AAA GGC TTT ATT GG-3') were used to amplify a 7.7-kb region using FideliTag Hotstart IT Mastermix (USB Corp.). The PCR conditions were 94°C for a 4-min initial denaturation, followed by 35 cycles consisting of 94°C for 30 s, 57°C for 30 s, and 68°C for 12 min, with a final extension of 15 min at 68°C. For second-round PCR, 3 μl of first-round products was amplified using 445T (4) and 7622R (5'-GGC CTG CAC TAC CGA AAT TCT GTC-3') to amplify a 7.1-kb region using the same conditions as in the first-round PCR. Products were separated on a 1% agarose gel.

RT-PCR. Total RNA was isolated from 200-μl plasma samples using MagMAX viral RNA (vRNA) isolation kits (Ambion, TX). RNA (5 μl) samples were analyzed in duplicate by quantitative reverse transcription-PCR (qRT-PCR) in comparison to standard in vitro synthesized XMRV gp70 Env RNA using Q6159R as the primer and Q6197R as the probe, as described elsewhere (11).

Analysis of cytokines by Luminescence. Sequential plasma samples were analyzed by a multiplex BioPlex flow-based assay described previously (18), performed at the core facility at the Baylor Institute for Immunology Research (Dallas, TX).

RESULTS

As a preliminary to in vitro inoculation, the growth of XMRV strain VP62 (4) was tested in rhesus macaque primary fibroblasts (Fig. 1a) using a reverse transcriptase assay (41), and XMRV was shown to replicate and produce Gag protein (Fig. 1b). Next, the presence of preexisting immunity to XMRV was tested in 25 plasma samples from rhesus macaques from the Yerkes colony, including the five animals used for the inoculation. As shown in Fig. 1c, no XMRV-specific antibodies were found, in contrast to a human plasma sample from a prostate cancer case harboring antibody reactive to XMRV p30 capsid (lane 1). Next, an initial set of three monkeys were inoculated with XMRV VP62, a molecular clone isolated from the prostate of a human patient (4), and followed for plasma viremia using quantitative RT-PCR (Fig. 2a). Among the three animals, plasma viremia was detected in two: one male (R11-10) with viremia starting on day 4 postinfection (p.i.), which
reached peak at 7,500 viral copies on day 7 and decreased to below the detection levels by day 14 (Fig. 2b); and a female (RYh-10), who showed a delayed and lower acute viral replication kinetics at days 14 to 20. The third animal (a male, RLq-10) never showed vRNA levels above the detection threshold. However, proviral DNA was detectable in all three animals during the initial 3 to 4 weeks p.i. in blood (Fig. 2c), confirming the susceptibility of macaques to XMRV infection.

FIG. 1. Evidence for XMRV replication in macaques ex vivo and in vivo. (a) Determination of XMRV reverse transcriptase activity in sequential supernatants collected from mock-infected primary rhesus fibroblasts (lane 1) and XMRV-infected primary rhesus fibroblasts (lanes 2 and 3) using a reverse transcriptase assay. (b) Detection of XMRV Gag in cell extracts (30 μg per lane) of the 10-day cultures described in panel a and detected using a rat anti-Gag monoclonal antibody. (c) Western blot analysis of preinfection plasma samples from rhesus macaques against supernatants from mock- and XMRV-infected DU145 cells (lanes 3 to 12). Lanes 1 and 2 represent a plasma sample from a positive human prostate cancer patient. CA, capsid.

FIG. 2. (a) Outline of the experimental monkey XMRV infection protocol and sample collections. LN coll, lymph node collection. (b) Plasma viral loads measured by real-time RT-PCR during the acute infection of three rhesus macaques. (c) Detection of XMRV Env provirus sequences in sequential PBMC samples prior to and during acute infection in the three original monkeys. Parallel amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was performed to ascertain the absence of amplification inhibitors within each sample. (d) Duplicate detection of XMRV Env provirus sequences in fractionated PBMC populations collected from days 3 to 7 postinfection. (e) XMRV provirus detection from PBMC collected from the two monkeys reinoculated with XMRV. (f) Quantitative RT-PCR amplification results from the plasma viral RNA collected from monkey RII-10 at necropsy. (g) Nested amplification of a nearly full-length XMRV genome (7.2 kb) from PBMC DNA of monkeys RII-10 and RLq-10 collected at day 18 pi.
Viral and proviral signals became consistently negative beyond 1 month p.i. until reinfection of two animals at day 158 (Fig. 2e). At that time, plasma viral RNA remained below the detection threshold, but transient proviral signals were detected, suggesting reinfection (Fig. 2e). More importantly, viremia was again detected in monkey RII-10 16 days after the immunization, suggesting that XMRV may be reactivated by immune activation and confirming the presence of replication-competent XMRV in this animal 4 months after its last exposure to the virus (Fig. 2f).

The lineage of blood cells replicating XMRV during acute infection was determined by the sorting of monocytes, B cells, and NK cells (Fig. 3). The frequencies of total T cells, proliferating T cells, and central memory and effector memory T cells were measured (Fig. 3a-g). Similarly, the frequencies of total B cells, proliferating B cells, and NK cells were also determined (Fig. 3h-j). These results provide evidence for the activation and proliferation of T cells and B cells in response to XMRV infection.

FIG. 3. Phenotypic analyses of monkey PBMC post-XMRV infection (day 0) and reinfection (day 158) and postimmunization with XMRV proteins (day 275) of the three monkeys followed longitudinally. (a) Frequencies of total T cells (CD3⁺). (b) Frequencies of proliferating Ki67⁺ T cells. (c) Frequencies of proliferating Ki67⁺ CD4⁺ total T cells. (d) Frequencies of proliferating Ki67⁺ CD8⁺ total T cells. (e) Frequencies of proliferating Ki67⁺ central memory CD4⁺ T cells (CD3⁺ CD4⁺ CD95⁻ CD28⁺). (f) Frequencies of proliferating Ki67⁺ effector memory CD4⁺ T cells (CD3⁺ CD4⁺ CD95⁻ CD28⁻). (g) Frequencies of proliferating Ki67⁺ central memory CD8⁺ T cells (CD3⁺ CD8⁺ CD95⁻ CD28⁺). (h) Frequencies of proliferating Ki67⁺ effector memory CD8⁺ T cells (CD3⁺ CD8⁺ CD95⁻ CD28⁻). (i) Frequencies of total B cells (CD20⁺). (j) Frequencies of proliferating Ki67⁺ B cells. (k) Frequencies of total NK cells (NKG2A⁺). (l) Frequencies of proliferating Ki67⁺ NK cells. Tcm, central memory T cells; Tem, effector memory T cells.

FIG. 4. Quantitation of soluble CD40 ligand (a) and IL-8 in monkey plasma (b) using the BioPlex technology.
CD4⁺ and CD8⁺ T cells, and NK cells from pooled peripheral blood mononuclear cells (PBMC) collected on days 3, 5, and 7 p.i. from each of the three animals using magnetic beads coated with antibodies to CD14, CD20, CD3, and CD4 or CD8, with NK cells being the remaining cells. The purity of the enriched subsets was verified by flow cytometry and ranged from approximately 60% for monocytes, 75% for B and CD8⁺ T cells, 80% for NK cells, and >93% for CD4⁺ T cells. XMRV DNA isolated from the enriched populations was amplified in duplicates and analyzed by electrophoresis (Fig. 2d). XMRV signal was consistently detected in CD4⁺ and CD8⁺ T cells and in NK cells. B cells were not consistently positive, and monocytes were mostly negative. These data mirrored the lymphotropism of XMRV reported in a cohort of chronic fatigue patients (17). To demonstrate the presence of nearly full-length XMRV genomic DNA, a 7.2-kb amplicon was amplified from day 18 p.i. PBMC DNA (animals RIL-10 and RLq-10) (Fig. 2g).

None of the infected animals showed any obvious clinical symptoms. Complete blood counts and sequential serum chemistry panels revealed only a mild elevation of creatine phosphokinase levels postinfection, which resolved rapidly and remained normal during the 9 months of monitoring. However, marked variations were observed in the phenotypic profiles of peripheral blood postinfection (Fig. 3). XMRV infection and reinfection were both accompanied by a marked increase in the frequency of circulating B and NK cells in blood. Even more remarkable was the increase in Ki67 expression by B cells and NK cells (Fig. 3i to l), as well as on memory CD4⁺ and CD8⁺ T cells (Fig. 3e to h), suggesting marked activation of lymphoid subsets following XMRV infection in vivo. Of note, immunization of the remaining two monkeys at day 275 with a cocktail of recombinant XMRV proteins in incomplete Freund’s adjuvant also induced a recrudescence of circulating B cells but a concomitant decrease in circulating NK cells (Fig. 3i and k).

It was assumed that infections would lead to detectable cytokine production, and, therefore, plasma samples corresponding to acute infection were subjected to multiplex flow-based analysis (data not shown). Out of 23 factors
tested, transient changes were noted for only the chemokine IL-8 and soluble CD40L and only in two out of the three monkeys, with a peak on day 4 p.i. (Fig. 4).

XMRV-specific cell-mediated responses measured both by short-term restimulation/flow analysis for gamma interferon (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor alpha (TNF-α) and by proliferation were essentially at background levels throughout the monitoring period, suggesting low levels of cell-mediated responses (data not shown). A caveat to these analyses was the limited availability of peptides spanning only the transmembrane Env protein and of whole virus as the stimulating antigens. More extensive analyses with a larger set of reagents will be needed to ascertain the magnitude of XMRV cell-mediated responses in this model. In contrast, antibody responses were clearly elicited after the initial infection (Fig. 5) and were boosted following reinfection as well as after immunization. However, the titers to XMRV antibodies rapidly decreased to a low level even after the second infection, indicative of a lack of sustained antigen stimulation of humoral responses, due to either poor release of XMRV from infected cells or the presence of immunosuppressive mechanisms (35). Of note, antibody responses to the envelope glycoprotein p70 and the transmembrane protein p15E dominated the early response although all structural proteins were recognized in Western blotting (28). Even though the antibody titers were relatively modest, plasma samples tested at the chronic phase (day 114 p.i.) showed evidence of neutralizing activity (Fig. 5c). Two additional animals were infected with XMRV and sacrificed during the acute infection period to obtain tissues representative of early virus dissemination. Serial sacrifice of XMRV-infected monkeys failed to demonstrate signs of pathogenicity in any of the five animals, two of which were followed for 9 months p.i. Immune activation was evident during acute infection, based on the formation of germinal centers in spleen and lymphoid organs (Fig. 6a and b), although compared to other lymphotropic retrovirus infections such as simian immunodeficiency virus (SIV), this activation was modest. Since XMRV was discovered initially linked with prostate cancer, the prostate was of particular interest as a potential site for pathogenicity. Histologic analyses showed mild to multifocal interstitial infiltrates of lymphocytes, neutrophils, and a few plasma cells in the prostate during acute infection (Fig. 6c and d), which appeared less pronounced in the two animals sacrificed at the chronic stage (Fig. 6e and f). However, prostates from age-matched control rhesus macaques also exhibited such findings without other evidence of prostate infection, arguing against a viral specificity for these observations (Fig. 6g and h).

In stark contrast to the absence of detectable XMRV genomic RNA in blood, detection of XMRV in situ was surprisingly abundant during acute infection as well as at the chronic stage. Lymphoid organs such as spleen, lymph nodes, and the gastrointestinal (GI) lamina propria all contained single round cells positive for XMRV by both immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) (Fig. 7). Moreover, the frequency of positive cells did not appear to vary dramatically between acute and chronic infection, except perhaps in the colon (Table 1), where increasing numbers of

![FIG. 6. Histological analysis of XMRV-infected monkeys in spleen and prostate using hematoxylin and eosin staining. (a and b) Splenic lymphoid architecture during acute XMRV infection on days 6 and 7 postinfection. Other images show prostate histology in monkeys acutely infected (c and d) and chronically (e and f) infected with XMRV as well as in uninfected age-matched controls (g and h).]
XMVR-positive cells were observed with time p.i. Using fluorescent IHC for XMRV and CD3 or CD4, the only cells identified to be positive for the presence of XMRV protein in the GI tract were CD3$^+$H11001 and CD4$^+$H11001 T cells (Fig. 7m and n), which was also the case for other lymphoid tissues such as spleen and lymph nodes, corresponding to the cell types infected in the blood during acute infection (Fig. 2d). The exception among these tissues was lung, which exhibited large numbers of XMRV-positive macrophages (Fig. 7k) throughout infection (Table 1) and few or no XMRV-positive T cells. There was an absence of signal by both IHC and FISH in the organs of control rhesus macaques that were not infected with XMRV (Fig. 7f and l and 8v and w).

To our surprise, when other tissues were investigated, completely distinct host cell lineages were observed to be XMRV positive. In the prostate, large foci of XMRV-positive acinar epithelial cells were detected by IHC during acute infection, suggesting rapid cell-to-cell transmission in glandular acini, while the surrounding stroma was uniformly negative (Fig. 8a and b). In contrast to acute infection, XMRV was no longer detected by IHC in prostate during chronic infection (Fig. 8c); although the virus was not eliminated, low-level nucleic acid signals were still observed using FISH (Fig. 8d). While not providing an etiological link to prostate carcinoma, these findings clearly indicate that the prostate constitutes an early target for XMRV. In contrast to findings in the prostate, XMRV-positive cells in the testes were far less abundant at any time and were exclusively interstitial, showing short strings of positive cells likely indicative of cell-to-cell transmission throughout infection (Fig. 8e to h). Other tissues of the reproductive tract showed evidence of replicating XMRV, such as the epididymis and seminal vesicle in male monkeys (Fig. 8j and k) and cervix and vagina in the female monkey (Fig. 8l and Table 1), suggesting the potential for sexual transmission. XMRV infection of interstitial cells was also detected in the pancreas during acute but not chronic infection. Other organs scored negative for XMRV by IHC (Table 1, brain, heart, kidney, bladder, gallbladder, etc.). These tissues were, however, not clear of XMRV since low-frequency nucleic acid signals were detected by FISH, suggesting generalized dissemination of XMRV (Fig. 8r to u) but lack of replication due perhaps to a paucity of suitable target cells, an unsuitable environment, or...
some form of active control of viral replication which remains to be determined.

DISCUSSION

The discovery of XMRV raises important questions about its potential as a human pathogen in light of established links of the MLVs, from which it is likely derived, to immunosuppression, neurological disorders, and cancers in mice and of established etiologic roles for similar diseases in humans by the retroviruses human immunodeficiency virus (HIV) and human T-cell leukemia virus (HTLV). We reasoned that nonhuman primates would constitute a model close to humans, with a comparable immune system, and this study is the first to report on the blood-borne infectivity, dissemination, and persistence of XMRV in animals known to express its natural functional receptor, XPR1. Our results not only show that XMRV was infectious for rhesus macaques but also demonstrate that after intravenous injection, XMRV established a persistently replicative infection in select tissues and organs even though circulation of free virus or infected cells outside the acute infection period appeared minimal or below detection limits in these healthy animals. It remains possible, though, that individuals with lowered immune functions, such as RNASEL dysfunction, may be more permissive for generalized infection and ongoing virus replication in tissues such as prostate, pancreas, and blood. Of note, XMRV appeared to respond to immune activation since low viremia (2,040 vRNA copies/ml) was detected in the plasma of one of the two monkeys administered XMRV proteins adjuvanted with incomplete Freund’s adjuvant on the day of sacrifice. The rapid resolution of the acute viremia suggests that innate mechanisms may largely contribute to containing the virus in the circulation (Fig. 1), a hypothesis supported by our lack of success in detecting antigen-specific cell-mediated responses to the virus and the rapid activation of NK cells. Infection with XMRV nevertheless caused activation and proliferation of several subsets of leukocytes (Fig. 2), which appeared amplified after reinfection, suggesting some level of anamnestic adaptive response in addition to innate responses, some of which also benefit from a type of memory (39, 40). Further analyses of cell-mediated immune responses to XMRV will clearly require additional study.

Most striking in this study was the organ-specific kinetics of viral replication. The initial targeting of prostatic epithelium and the reproductive tract suggests that the enhancement of XMRV replication in response to androgen stimulation in vitro may represent a physiological mechanism (5) and may reflect lack of APOBEC3G expression in this tissue (2) and the reproductive tract suggests immune control of the virus as well, which is muted in the immuno-privileged environment of testes but not in the immune-competent prostate. Furthermore, continued replication in lymphoid organs may be secondary to localized immune activation, as suggested by other investigators (17), a

TABLE 1. Semiquantification of XMRV signal in relation to tissue and time postinfection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Relative XMRV signal at the indicated time postinfection (animal, gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 dpi (RLm-1, M)</td>
</tr>
<tr>
<td>Spleen</td>
<td>++</td>
</tr>
<tr>
<td>Axillary LN*</td>
<td>++</td>
</tr>
<tr>
<td>Inguinal LN</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>++</td>
</tr>
<tr>
<td>Mediastinal LN</td>
<td>++</td>
</tr>
<tr>
<td>Pelvic LN</td>
<td>+++</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>+++</td>
</tr>
<tr>
<td>Liver</td>
<td>Background</td>
</tr>
<tr>
<td>Jejunum</td>
<td>++</td>
</tr>
<tr>
<td>Ileum</td>
<td>+</td>
</tr>
<tr>
<td>Colon</td>
<td>++</td>
</tr>
<tr>
<td>Pancreas</td>
<td>+++</td>
</tr>
<tr>
<td>Brain stem</td>
<td>-</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>-</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-</td>
</tr>
<tr>
<td>Heart</td>
<td>-</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>-</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>-</td>
</tr>
<tr>
<td>Testes/ovaries</td>
<td>+++</td>
</tr>
<tr>
<td>Prostate</td>
<td>+++</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>+++</td>
</tr>
<tr>
<td>Epididymis</td>
<td>-</td>
</tr>
<tr>
<td>Cervix</td>
<td>NA</td>
</tr>
<tr>
<td>Vagina</td>
<td>NA</td>
</tr>
</tbody>
</table>

* dpi, days postinfection; F, female; M, male; NA, not available. Number of fields staining positive with IHC per ten fields (magnification, ×20); --, no positive cells; +, 1 to 4 cells; ++, 5 to 10 cells; ++++, more than 10 cells.

b Determined by FISH.

Ovariess.

d LN, lymph node(s).
FIG. 8. Detection of XMRV infection in nonlymphoid organs in situ using IHC and FISH. (a to d) Prostate samples from acutely (a and b) and chronically infected monkeys (c and d, showing IHC and FISH, respectively). (e to h) Testis samples from acutely (e and f) and chronically (g and h) infected monkeys. (i) Pancreas from collected during acute XMRV infection. (j) Epididymis collected at 5 months postinfection. (k) Seminal vesicle collected at 291 days postinfection. (l) Cervix collected at 291 days postinfection. (m) Uninfected control vagina. (n to u) XMRV FISH detection on samples from monkey RYh-10 at 291 days postinfection including lung (n), spleen (o), jejunum (p), colon (q), heart (r), liver (s), vagina (t), and brain stem (u). (v) Uninfected control liver. (w) Uninfected control vagina. Signal was detected even in brain and heart in contrast to negative IHC findings.
finding that needs to be explored in more detail. Presence of XMRV in the lower reproductive tract in both male and female monkeys is consistent with the potential for sexual transmission. In addition, we have previously demonstrated XMRV in expressed prostatic secretions in humans (11) as well as enhanced infectivity for prostate epithelial cells in the presence of human semen or cationic amyloid fibrils derived from prostatic acid phosphatase, the most abundant protein in human semen, which has been shown to promote transmission of HIV and XMRV in vitro (11, 23, 29).

Another interesting observation was the selective infection of defined cell lineages by XMRV in different organs: lymphocytes in lymphoid organs, macrophages in lung, and epithelial or interstitial cells in other IHC-positive organs. To our knowledge, this viral behavior appears specific to this virus, and ongoing investigations are directed at the elucidation of mechanisms underlying such observations. The permissiveness of human cell lines to XMRV in vitro appears rather extensive, perhaps due to the widespread presence of the XPR1 receptor (37), and, thus, modulation of XMRV replication in vivo may rely on other mechanisms, such as RNAseL activity, activation of type I interferons, or localized expression of restriction by factors such as APOBEC3 and tetherin (2, 4, 10, 27). In this regard, APOBEC3G is absent in prostate cancer lines, suggesting that such cells provide an optimal environment for XMRV infections (2, 27). The early targeting of prostate epithelium by XMRV suggests that the virus finds it a preferential site, and even though our studies showed lack of continued replication in vivo, chronic persistent infection could play a role in disrupting homeostasis and promoting cell cycle dysfunction (22). Since the completion of this study, an additional four reports suggested that XMRV detection in specimens collected from human patients might be the result of laboratory contamination (12, 25, 30, 33), further fueling the controversy as to whether XMRV is a human retrovirus and questioning its pathogenesis, and the efficacy of therapeutic agents (31, 36) controlled.

ACKNOWLEDGMENTS

We are indebted to the staff of the Yerkes National Primate Research Center for their outstanding care of the animals and support for this project, to John Connolly (Baylor Institute for Immunology Research, Dallas, TX) for the performance of the BioPlex cytokine detection, and to Priscilla Swanson and Ka-Cheung Luk (Abbott Diagnostics) for development of recombinant proteins used to characterize the antibody response to XMRV.

These studies were made possible by support from Abbott Diagnostics and the NCRR support to the Yerkes NPRC (DKR000165), by grants to R.H.S. from NIH (NCI; CA103943), the U.S. Department of Defense (W81XWH-07-1-0338), and Lea Bank, by the Charlotte Geyer Foundation (to R.H.S. and E.A.K.), and by the Milton and Tamar Maltz Family Foundation (to E.A.K.).


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