MULTIPLE DRUG RESISTANCE MUTATIONS IN HUMAN IMMUNODEFICIENCY VIRUS IN SEMEN BUT NOT BLOOD OF A MAN ON ANTIRETROVIRAL THERAPY
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ABSTRACT
The concept that the male reproductive tract harbors isolated reservoirs of human immunodeficiency virus (HIV) infection has now been widely accepted. The significance of semen viral burden to sexual transmission of HIV is obvious; however, its contribution to disease progression is unknown. We report a case study that demonstrates the emergence of resistance-conferring mutations to antiviral therapy in infected seminal leukocytes from a man with asymptomatic prostatitis associated with leukospermia. This finding demonstrates the potential importance of male reproductive tract organs to the development of therapy resistance in HIV-infected men.


We1,2 and others3,4 have shown that the male reproductive tract is an isolated reservoir of human immunodeficiency virus (HIV) infection which responds differently from blood to antiviral therapy. The profound decrease in blood plasma viral burden in men on multidrug antiviral therapy has produced hope that HIV infection may be eradicated.3 Unfortunately, the rapid appearance of viral substrains resistant to therapy remains a significant obstacle.6 Mathematical modeling reveals the need for two compartments of HIV infection to account for the rapid rate of emergence of drug-resistant mutations.7 The model assumes one compartment is larger with a higher drug level (eg, the vascular/lymphatic systems) and the other is smaller with a lower drug level that permits the production of escape mutants. We report a case study that supports the theory that semen-producing organs, reported to have a lower concentration of some antiretrovirals,8 can serve as the smaller compartment.

CASE REPORT
Paired blood and semen specimens were obtained from an asymptomatic HIV-infected man on antiviral therapy including a protease inhibitor for 4 years. The man was enrolled in our longitudinal study (reviewed and approved by the internal review board of the Beth Israel Deaconess Medical Center) and had a history of recurrent, asymptomatic prostatitis, detected by leukospermia and a boggy prostate on digital rectal examination. Each episode resolved after a 30-day course of oral ciprofloxacin. The blood and semen specimens analyzed were obtained during such an episode.

Seminal and blood plasmas were separated from cells by centrifugation and analyzed for viral burden by measuring viral RNA copies with the Nuclisens assay (Organon, Durham, NC).9 The frequency of infected cells in semen and blood was measured by our standard end-point dilution assay.10 The relatedness of viral subspecies in each specimen was determined by genetic analyses of HIV protease gene sequences. Seminal and blood plasma virus ribonucleic acids (RNAs) were purified by the method of Boom,11 and protease genes amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) followed by cloning and sequencing as described.1,2 Nonsperm semen cells were isolated by Optiprep gradients.10 Cellular deoxyribonucleic acids (DNAs) were purified for PCR amplification of protease gene sequences which were then cloned and sequenced as report-
TABLE I.  Protease amino acid sequences in blood and semen human immunodeficiency virus

|     | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  | 46  | 47  | 48  | 49  | 50  | 51  | 52  | 53  | 54  | 55  | 56  | 57  | 58  | 59  | 60  | 61  | 62  | 63  | 64  | 65  | 66  | 67  | 68  | 69  | 70  | 71  | 72  | 73  | 74  | 75  | 76  | 77  | 78  | 79  | 80  | 81  | 82  | 83  | 84  | 85  | 86  | 87  | 88  | 89  | 90  | 91  | 92  | 93  | 94  | 95  |
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| hxb2 | PQTVLWQRPLVTVIKGGQLKEALLDGTADTVLLEEMSLPGRNKPKMI | GGI | GGF | F | KV | R | Q | D | Q | I | L | E | I | CG | HKAI | GTV | L | V | O | PT | PVN | I | GRL | LTQ | I | GCT | L | NF |

Five to twenty copies of human immunodeficiency virus ribonucleic acid (plasmas) and provirus deoxyribonucleic acid (cells) were amplified, cloned, and sequenced as described. The amino acid sequence for protease in the wild-type reference virus, hxb2, is listed at the top and bottom of the table. Amino acid sequences for each semen and blood virus clone are indicated by a dot where there is agreement with hxb2, and by the letter corresponding to the amino acid in substituted positions. Shaded columns indicate sites of protease inhibitor resistance conferring mutations.
Genetic analyses of HIV protease genes revealed three distinct groups (Table I). The residual virus particles in the blood and seminal plasmas were highly homologous, and together comprised one group with few protease gene mutations. The provirus protease genes in the infected blood cells formed a second homogeneous group which contained several mutations not found in the plasma viruses, indicating the blood cells were not the source of the residual plasma viruses.

In contrast to the plasma viruses and the blood cell proviruses, the protease genes in infected semen cells were highly heterogeneous (Table I), indicating they resulted from multiple independent rounds of infection, since the error rate of HIV reverse transcriptase leads to an average of one mutation per cycle of infection.6,7 The patient’s occult prostatitis probably fueled the infection,12 resulting in both increases in infected semen leukocytes and mutations in HIV protease. The homology between the semen cell clones and the seminal plasma virus with a resistance mutation suggests the infected semen cells were the source of at least some of the seminal plasma virus. In all, the semen provirus clones contained 12 of the 16 mutations reported to confer resistance to the patient’s protease inhibitor.6

COMMENT

In contrast to prior semen HIV studies,3,4 this report describes protease genes in both plasma viruses and infected cell proviruses from paired blood and semen specimens obtained from a man on highly aggressive antiviral therapy (HAART). The increased frequency of resistance-conferring mutations in the HIV protease genes in infected semen cells in this man with well-controlled HIV disease provides proof-of-principle of the mathematical model’s prediction of therapy-resistant escape mutants replicating in a smaller compartment of infection.7

This case study highlights the complex nature of residual HIV disease in men on HAART and raises several important concerns. First, semen viral burden may be highly discordant with blood viral burden, in keeping with previous reports.2 Therefore, blood sampling alone leads to the possibility that men with undetectable blood plasma viral RNA copies may wrongly assume that their HIV disease is also undetectable in semen and unwise abandoning safe sexual practices. Second, asymptomatic reproductive tract infections in HIV-infected men may contribute significantly to both the burden of HIV in semen, as also reported for urethritis in African men,12 and the emergence of therapy escape mutants. Not only may such escape mutants ultimately lead to resistant viral subspecies in blood, but they may also be sexually transmitted and should safe sexual practices be abandoned. This may significantly escalate the propagation of resistant strains of HIV. Third, the ability of current antiviral drugs to penetrate and achieve virucidal levels in male reproductive tract tissues is in urgent need of evaluation. Although levels of zidovudine in seminal plasma approach those in blood plasma,8 strategies aimed at both blood and semen virus are clearly needed. Fourth, regularly monitoring reproductive tract health and semen viral burden in men with HIV disease may be important. The occult prostatitis in the man described in this case study significantly increased the HIV burden in seminal cells, suggesting that inflammation in semen-producing organs may be important in elevating seminal viral burden.

Studies are urgently needed to determine the incidence and prevalence of leukospermia in HIV-infected men, and the effect on overall HIV mutation rates of antibiotic treatment aimed at reducing the leukospermia.

REFERENCES


