High Prevalence of Antibodies against HERV-K10 in Patients with Testicular Cancer but not with AIDS[1]

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Abstract

Human endogenous retrovirus K10 (HERV-K10) *env* and *gag* expression has been detected in placenta, embryonic tissue, and cell lines. By transfection, these sequences have been expressed in insect cells and developed into serological assays, revealing HERV-K10 antibodies in patients with testicular cancer. Patients with AIDS are at an increased risk for testicular cancer and frequently reactivate latent infections. We postulated that HERV-K10 seroprevalence might be increased with HIV infection or AIDS.

Stored, frozen serum samples from 52 patients with testicular cancer (8 patients with HIV and 30 patients with samples near the time of diagnosis) and 84 controls (40 patients with HIV) were diluted 1:40 and tested by immunofluorescence against SF158 cells transfected with HERV-K10 *env* [ENV1.9(+)] or *gag* (pACGAG). Seroprevalence rates were compared cross-sectionally in cases and controls, excluding those with indeterminate results (3 of 30 cases and 7 of 84 controls), and also were examined longitudinally in the cases before or after diagnosis of testicular cancer.

Seroprevalence to HERV-K10 Env or Gag was 17 of 27 testicular cancer patients (63%) around the time of diagnosis, compared to 4 of 77 controls (5%; $P < 0.0001$). Seroprevalence was similar (50% to 60%) with seminoma, teratocarcinoma, or embryonal carcinoma, and it was not increased with HIV infection in either cases (33%) or controls (3%). HERV-K10 antibodies were detected in 12 of 19 cases (63%) more than 6 months before seminoma diagnosis, as well as in four cases with residual or recurrent malignancy more than 1 month after initial diagnosis.

Thus, HERV-K10 antibodies are detected frequently with testicular cancer and seem to resolve rapidly with effective therapy of the malignancy. Antibody reactivity also occurs in ~5% of controls, perhaps because of nonspecific or cross-reactive epitopes. HIV and AIDS were not associated with HERV-K10 antibodies, thus, leaving their higher risk of testicular cancer unexplained.

Introduction

HERV[3] is the term given to sequences of human DNA that are homologous to infectious retroviruses (1). Unlike the two families of exogenous human retroviruses, the human T-cell lymphotropic viruses, and HIVs, nearly all HERVs are defective, with no evidence for pathogenic effects or horizontal transmission. HERV-K10, however, has largely intact coding regions. Moreover, expression of HERV-K10 *gag* and *env* sequences has been found in human placenta, embryonic tissue, cell lines, and malignant germ cell tumors (2, 3). Transfection of HERV-K10 *gag* and *env* into insect cells has enabled the development of prototype antibody assays that have revealed high prevalence rates of anti-HERV-K10 antibodies in patients with testicular and other germ cell carcinomas (4, 5).

We and others (6, 7) have recently noted an increased risk of seminoma among persons with AIDS. We postulated that severe immune deficiency, such as AIDS, might result in the activation of HERV-K10 and lead to an increased risk of seminoma or other malignant germ cell tumors. We evaluated the relationship of HERV-K10 antibodies to testicular cancer, as well as the possibility that HERV-K10 antibody prevalence might be increased with HIV infection or AIDS.

Materials and Methods

Sera or plasma samples, stored at or below -20°C for up to 20 years, were selected from testicular cancer patients (8) and from testicular cancer cases and controls in three prospective cohort studies of persons at high risk of AIDS [the MACS (7), the Multicenter Hemophilia Cohort Study, and the Washington and New York Men's Research Study (9)] and from families prone to testicular cancer (10). Eight of the 52 cases with stored sera were infected with HIV (7). Additional controls who had similarly frozen sera were selected from healthy volunteer blood

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[3] The abbreviations used are: HERV, human endogenous retrovirus; MACS, Multicenter AIDS Cohort Study; HCG, human chorionic gonadotropin; AFP, α-fetoprotein.

Table 1 Prevalence of HERV-K10 antibodies among patients with testicular cancer and controls

Group (no. tested)[a]	HERV-K10 antibody prevalence				
	Env antibodies (%)		Gag antibodies (%)		Env or Gag (%)
	Indeterminate	Positive	Indeterminate	Positive	Positive[b]
Testicular cancer (30)	3	16 (59%)	7	6 (23%)	17 (63%)
By HIV status					
HIV positive (6)	0	2 (33%)	0	0	2 (33%)
HIV negative (24)	3	14 (67%)	7	6 (35%)	15 (71%)
By histopathology					
Seminoma (19)	0	10 (53%)	3	3 (19%)	11 (58%)
Teratocarcinoma (6)	0	3 (50%)	0	1 (17%)	3 (50%)
Embryonal carcinoma (5)	0	3 (60%)	1	2 (50%)	3 (60%)
Controls					
All groups (84)	7	3 (4%)	7	1 (1%)	4 (5%)
By group					
AIDS controls (21)[c]	1	0	1	0	0
HIV-positive controls (19)[c]	3	1 (6%)	3	0	1 (6%)
HIV-negative controls (8)	1	0	1	1 (14%)	1 (14%)
Cancer-prone families (16)	2	1 (7%)	2	0	1 (7%)
Male blood donors (20)	0	1 (5%)	0	0	1 (5%)

[a] Only 30 of the 52 testicular cancer cases had specimens collected between 180 days before and 30 days after the date of diagnosis. Longitudinal results that included data from all 52 cases are presented in the text. HIV-positive controls included 39 homosexual men and one man with hemophilia. HIV-negative controls were all homosexual men. Cancer-prone families included renal adenocarcinoma cases and their first-degree relatives, as well as first-degree relatives of testicular cancer cases (10, 11).

[b] For overall prevalence estimates, samples were excluded only if both Env and Gag results were indeterminate.

[c] AIDS defined as HIV positive with $CD4^+$ lymphocyte count <150 cells/μl. Other HIV-positive controls had higher $CD4^+$ lymphocyte counts at the time of phlebotomy.

donors and from families prone to polythelia and renal cell carcinoma (11). Sera were tested in two batches. The first batch included one sample/subject (closest to the date of diagnosis for cases). The second batch included longitudinal samples on subjects whose sample in the first batch was positive, as well as additional HIV-matched controls from MACS. All 20 of the HIV-positive controls from the Washington and New York Men's Research Study have developed an AIDS-defining opportunistic illness; at the time of phlebotomy, their median $CD4^+$ lymphocyte count was 64.5 cells/μl, with 19 of 20 below 150 cells/μl. The six HIV-positive testicular cancer cases (all from the MACS) had a median $CD4^+$ lymphocyte count of 305 cells/μl (range, 150–776). The MACS controls were matched to the testicular cancer cases by HIV status and study visit. The MACS HIV-positive controls had a median $CD4^+$ lymphocyte count of 424 cells/μl (two below 150 cells/μl) at the time of phlebotomy. Six controls have developed an AIDS-defining opportunistic illness.

As reported previously (4, 5), sera or plasma samples were tested for HERV-K10 Env or Gag antibody reactivity using indirect immunofluorescence against SF158 cells that had been transfected with the ENV1.9(+) or pACGAG fragments. Wild-type baculovirus-infected SF158 cells served as controls. Positive samples, those that demonstrated specific immunofluorescence at a 1:40 dilution, were titered with further 4-fold serial dilutions. Samples with high background reactivity were considered indeterminate; those with indeterminate results against both Env and Gag were excluded from seroprevalence calculations. Aliquots of selected samples also were tested for levels of HCG and AFP by a commercial reference laboratory using licensed kits. In addition to longitudinal HERV-K10 seroprevalence rates among cases, cross-sectional results for samples collected near the time of diagnosis (30 of 52 cases) were compared with those of the control groups. Differences in seroprevalence were evaluated with a two-sided Fisher's exact test.

Results

Cross-sectional HERV-K10 seroprevalence rates are presented in Table 1. The prevalence of antibodies to either K10 Env or Gag was 17 of 27 testicular cancer patients (63%) who had evaluable results on sera that were drawn from 180 days before to 30 days after cancer diagnosis. Only two of the six HIV-infected testicular cancer cases (33%) had HERV-K10 antibodies, but this was not significantly different from the 71% seroprevalence observed among HIV-uninfected cases ($P = 0.10$). Five of the six HIV-infected testicular cancer patients had seminoma, including the two who were positive for K10 antibodies. HERV-K10 seroprevalence was similar (50% to 60%) in the three histopathological types of testicular cancer (Table 1).

Among 77 controls with evaluable results, four (5%) had HERV-K10 antibodies, significantly lower than the seroprevalence in the cancer cases ($P < 0.0001$). The seropositive controls were approximately equally distributed among the various control groups (Table 1). The one HERV-K10-positive volunteer blood donor was a young, white man; classical serological markers of testicular cancer were well within the normal range, including HCG (<2 mIU/ml; normal, <5 mIU/ml) and AFP (<2.5 ng/ml; normal, <8.1 ng/ml). In the three members of a black family prone to renal adenocarcinoma who had indeterminate or positive HERV-K10 antibodies, HCG levels were normal (<2 mIU/ml), but AFP levels were high-normal to slightly elevated (7.1–9.7 ng/ml). These three family members were ages 48–68 and included one with renal carcinoma, one with polythelia, and one with neither condition. The other two HERV K-10 seropositive controls had insufficient sera for the evaluation of tumor markers.

Considering the HERV-K10 assays in more depth, 8–20% of the sera had indeterminate results due to high background, nonspecific reactivity (Table 1). The assay for Env had higher sensitivity (59% positive among cases) but lower specificity (4% positive among controls) than did the Gag assay (23% among cases, 1% among controls). Similarly, antibody titers

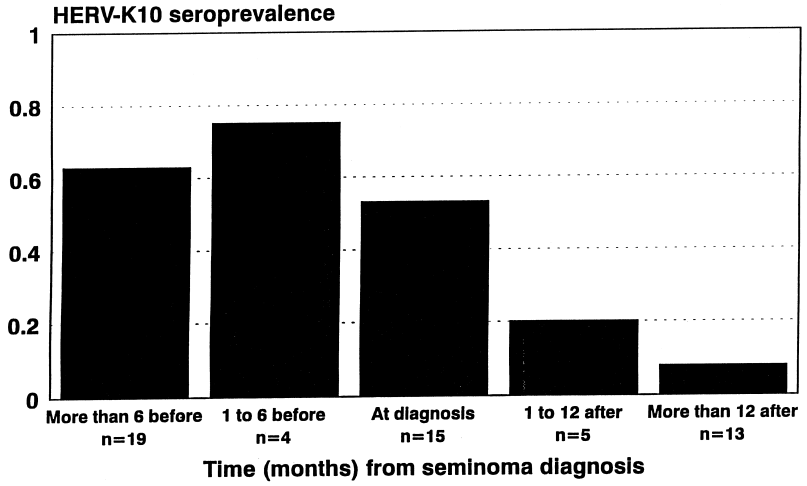

Fig. 1. Prevalence of antibodies against HERV-K10 in sera collected at various times before, at, or after the diagnosis of seminoma. Sera were considered positive if antibodies were detected to either Env or Gag antigens of HERV-K10.

tended to be higher against Env than against Gag. Among the HERV-K10 seropositive testicular cancer cases, median antibody titers were 1:1280 (range, 1:80–1:10240; $n = 22$) against Env, compared with 1:160 (range, 1:80–1:5120; $n = 6$) against Gag.

Using sera that had been collected and frozen prospectively, antibodies against HERV-K10 Env or Gag were detected >6 months before diagnosis in 12 of 19 seminoma cases (63%) and likewise were detected 1–6 months before diagnosis in 3 of 4 seminoma cases (75%; Fig. 1). One of five seminoma cases (20%) was positive 1–12 months after diagnosis, and one of 13 cases (8%) was positive thereafter. Among the nonseminoma cases, 0 of 11 with prediagnostic sera had HERV-K10 antibodies; but, six of nine nonseminoma cases (67%) were seropositive within 2 months of diagnosis, as were three of six (50%) and one of three cases (33%) who were tested 1–12 months and >12 months, respectively, after diagnosis. Residual or recurrent malignancy was documented in four of the six cases that were seropositive after diagnosis. The fifth case was last tested on completion of radiation therapy 61 days after diagnosis but, thereafter, he was lost to follow-up. The sixth case was seropositive 16 months after orchiectomy for stage I teratoma and remains free of tumor more than 15 years later. None of the six cases who had HERV-K10 antibodies after diagnosis were infected with HIV. Appearance of antibodies before diagnosis and disappearance after successful therapy was observed in those individual cases that had closely spaced samples (data not shown).

Discussion

The etiology and pathogenesis of testicular cancer is largely unknown. Cancer risk is increased in certain congenital conditions, including Kleinfelter syndrome, hypospadias, polythelia, and especially cryptorchidism, which accounts for 10% of testicular cancer cases (10, 12, 13). Because surgical correction of cryptorchidism before age 10 markedly reduces the cancer risk, local dysgenesis or other conditions within the testis clearly are reversible (12). Both testicular cancer and cryptorchidism occur excessively and have been increasing in incidence among white men and those of higher socioeconomic status (12). Although a number of occupations have been associated with testicular cancer, no specific exposure has been identified. Similarities in the epidemiology of testicular cancer and Hodgkin's disease in young men have been noted and have raised the possibility that herpes viruses might contribute to testicular cancer risk (14, 15).

Men with AIDS have a nearly 3-fold increased risk of testicular cancer (6). Because a hallmark of HIV infection and AIDS is the reactivation of certain latent viruses and other infections (often those that are readily controlled by intact cellular immunity), we postulated that HERV-K10 may become derepressed with HIV-related immune deficiency, leading to the expression of *env* or *gag* and to the development of antibodies. Our data did not support this hypothesis. The prevalence of HERV-K10 antibodies was only one (3%) of 36 HIV-infected men, not different from the one (5%) found among 20 healthy HIV-uninfected male blood donors. None of 20 HIV-infected subjects with very severe immune deficiency had antibodies against either HERV-K10 protein.

As is true for HCG and AFP, which are the classical markers of testicular cancer, the HERV-K10 *env* and *gag* genes probably are repressed in most adult cells and function only during embryonic development, if ever. On the off chance of a strong inheritance pattern in the regulation of these HERV-K10 genes, we included as controls unaffected members of families that are prone to testicular or renal cell carcinomas. HERV-K10 antibody prevalence in these family members was low and similar to the low prevalence seen in the other control subjects. The lack of anything in common among the four seropositive control subjects suggests that their antibody reactivity may have been nonspecific. In addition, although these sera had been stored frozen under good conditions, indeterminate results due to high background reactivity were found in 9% of the Env and 12% of the Gag slides.

We did confirm a high prevalence of HERV-K10 antibodies at the time of testicular cancer diagnosis. Seminoma cases frequently had such antibodies for 6 months or more before diagnosis. Despite its relatively high sensitivity of ~60%, testicular cancer is such a rare disease even among white men during peak ages (13/100,000 per annum from age 20–34) that the positive predictive value of HERV-K10 would be extremely low. In addition, HERV-K10 antibodies in four control subjects yields a specificity of ~95%. Thus, these assays may be very useful for research, but they would not be appropriate for large-scale screening.

In summary, HERV-K10 is an endogenous human retro-

virus, which means that it is a constitutive part of the genome of all human cells. HERV-K10 *env* and *gag* genes seem to be expressed in embryonic, placental, and testicular cancer tissue. Moreover, the proteins produced by these genes are antigenic, and the resulting antibodies can be found frequently in the sera of testicular cancer patients. As with classical markers of testicular cancer, HERV-K10 antibodies resolve rapidly with effective treatment of the testicular cancer, supporting the theory that HERV-K10 expression is limited to the tumor. Whether expression of HERV-K10 contributes to the pathogenesis of testicular cancer and whether HERV-K10 serology might be useful for monitoring patients who have had testicular cancer or for other purposes remains to be seen.

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