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Characterization of the Antibody Response Specific for the Human Endogenous Retrovirus HTDV/HERV-K

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Differentiated human teratocarcinoma cell lines produce the human teratocarcinoma-derived virus (HTDV) particles encoded by the human endogenous retrovirus sequence HERV-K. We screened almost 2,000 human sera for antibodies against this endogenous human retrovirus, HTDV/HERV-K. Specificity of the immunofluorescence reactions using particle producing teratocarcinoma cells was confirmed by immunoelectron microscopy of ultrathin frozen sections. Immunoblot analyses using lysates of HTDV-producing cells revealed a 80-kDa HERV-K Gag precursor and a 90-kDa putative viral Env protein after incubation with positive sera. No processed Gag protein could be observed. Virus-specific bands were not detected in lysates of nonproducing cells. High antibody titers were found in about 60% of male patients with germ cell tumors. Antibody reactivity declined after tumor removal. In healthy blood donors, anti-HTDV reactivity was found only at low titers in a small percentage (3.9%) of individuals. A slightly elevated but statistically significant percentage of HTDV positivity was also observed for sera of pregnant women, whereas human immunodeficiency virus-positive individuals exhibited no peculiarity compared to normal blood donors. Our results provide evidence that HTDV particles are expressed in vivo and that the immune reaction against HTDV/HERV-K is specific for defined viral proteins.

HTDV (human teratocarcinoma-derived virus) was originally detected by electron microscopy in cell lines that were established from human teratocarcinomas (3, 19). They resemble type C retroviruses with respect to morphology and the budding process at the plasma membrane of the virus-producing cell (1). A unique feature of HTDV is the lack of an electron-lucent space between core and envelope. Virus particles remain at the cell membrane, arrested in late budding stages, and free mature particles with condensed cores are only rarely observed (1). As infectivity of HTDV has so far not been demonstrable (22), it was concluded that HTDV is a defective human endogenous retrovirus (HERV). We have recently shown that HTDV is encoded by the endogenous retroviral sequence HERV-K (2, 24).

HERV-K is a retroviral sequence family comprising about 50 copies in the human genome (32). Prototype HERV-K is the only known human endogenous retrovirus with open reading frames for all structural and enzymatic proteins (23, 30, 33). In addition, the translation product of a central open reading frame is localized in the nucleolus, which is reminiscent of the human immunodeficiency virus (HIV) *rev* gene product described previously (25).

HERV-K must have entered the human genome about 30 million years ago (38, 40), as these sequences are not found in New World primates. Although full-length mRNA expression was detectable in many tissues (29), protein and particle expression has been demonstrated only in cell lines established from human teratocarcinomas (2, 3, 19, 35). To date, HERV-K is the only known HERV sequence that encodes virus-like particles. Although particles that are morphologically indistinguishable from HTDV have been repeatedly observed in human placentas (15, 27, 44)), there is no definitive evidence that they are encoded by HERV-K genes.

In animals, the existence of natural antibodies to endogenous retroviruses is well established (5, 8, 37). Several reports have described a possible human antibody reactivity against endogenous retroviruses. In some studies, antibodies were detected by virtue of a cross-reaction to exogenous human retroviruses or to animal retroviruses (18, 43). Other authors have described reactivities against recombinant proteins or against synthetic peptides of known human endogenous retroviral sequences (4, 6, 34, 45). However, the exact specificity of those antibodies is still unclear. It remains to be determined whether these antibodies are in fact elicited by the corresponding retroviral antigens or whether they represent cross-reactivities to other, as yet unknown retroviruses or to other antigens. Cross-reactivity is a well-known phenomenon among Gag proteins of different retroviruses. Furthermore, it has been shown repeatedly that antibodies against retroviral envelope proteins may be directed against the carbohydrate moiety of the glycoprotein, which is host cell dependent and therefore not virus specific (21, 28).

Antibodies recognizing recombinant Gag proteins of HERV-K have been observed in patients with seminomas and teratocarcinomas (35). Antibodies against recombinant protein or against synthetic peptides of HERV-K Env were found in sera of patients with repeatedly elevated serum neopterin concentrations (45) and with testicular tumors and lymphomas and in HIV-positive persons as well as in pregnant women (6, 26). However, direct binding of natural antibodies to HTDV particles has not yet been demonstrated. We therefore investigated whether a natural human antibody reactivity against HTDV exists.

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MATERIALS AND METHODS

Cell culture. The HTDV-producing GH cell line was established at our institute in 1979 from a patient with a teratocarcinoma (22). M7 cells producing

baboon endogenous virus (BaEV) and NRK cells producing simian sarcoma virus SSV/SSAV were a gift from R. Löwer (Paul-Ehrlich-Institut), and the cell line GR, infected with mouse mammary tumor virus (MMTV), was kindly provided by H. Ponta (Kernforschungzentrum Karlsruhe GmbH, Karlsruhe, Germany). All other cell lines were obtained from the American Type Culture Collection. Cell cultures were grown in CMRL medium (13) supplemented with 5% fetal calf serum, 2 mM glutamine, and antibiotics. With the exception of the GH cell line, all cultures were split once weekly between 1:4 and 1:10, using trypsin. The cell line GH was split 1:3 every 10 days without trypsin, with cells being removed from the culture flask by vigorous shaking and pipetting (22).

Cloning, expression, and purification of HERV-K Gag and generation of antiserum. A full-length HERV-K *gag* cDNA clone (pcGU1; nucleotides [nt] 822 to 3626) was isolated from a randomly primed GH cDNA library (42a), using a HERV-K *gag* probe (25). Plasmid pcGU1 was characterized by restriction enzyme analysis and sequenced by the dideoxy-chain termination method. All primers were purchased from Eurogentec (Belgium) or produced on an ABI 380B DNA synthesizer in our institute. The numbering of nucleotides is based on the HERV-K type 2 sequence as described previously (25), including an inserted G at position 1749 (30) and revealing a complete HERV-K *gag* open reading frame. For prokaryotic expression of HERV-K Gag, fragment GU1 (nt 1117 to 3145) was derived from pcGU1 by PCR amplification using synthetic oligonucleotides Mut Bam F (5′-GGGTGATAATGGGGGATCCTAAAAGTAAAAT T-3′, nt 1104 to 1135) and Mut Bam R (5′-GGGGGCTCCCCTGGATCCAGA GAGACT-3′, nt 3157 to 3131), containing *Bam*HI restriction sites (underlined). After PCR (5 min at 94°C, 45 s at 94°C, 2 min at 55°C, and 3 min at 72°C for 10 cycles; 45 s at 94°C, 2 min at 60°C, and 3 min at 72°C for 15 cycles; and 10 min at 72°C), the *Bam*HI-digested HERV-K *gag* fragment was cloned in frame into the *Bam*HI restriction site downstream of the histidine hexamer in pET15b (Novagen). The fusion protein was expressed in *Escherichia coli* and isolated from the membrane fraction of induced *E. coli* BL21 (DE3) (Novagen) after solubilization in buffer A (8 M urea, 20 mM Tris-HCl [pH 7.9], 1 mM EDTA) and purification by Ni chelate affinity column chromatography under denaturing conditions as instructed by the manufacturer (Novagen). Goats were immunized with 100 μg of protein in Freund's incomplete adjuvant. Animals received boosters twice before antiserum was drawn.

Immunocytochemistry. For immunocytochemical staining, cells were grown on slides or coverslips, fixed with 2% freshly prepared formaldehyde in phosphate-buffered saline (PBS), permeabilized with 1% Triton X-100 (Merck, Darmstadt, Germany) in PBS, and treated with 1% bovine serum albumin (Sigma, Deisenhofen, Germany) to block nonspecific binding sites. During screening, human sera were routinely incubated at a dilution 1:100 for 60 min at 37°C. As a positive control and for double-labelling experiments, the goat antiserum against HERV-K Gag protein was used at a dilution of 1:800. After being washed in PBS, the cells were incubated for 30 min with either fluorescein isothiocyanate- or alkaline-phosphatase-conjugated anti-immunoglobulin G (IgG) secondary antibodies (Organon Teknika, Heidelberg, Germany). After renewed extensive washing, enzyme reaction was performed for alkaline phosphatase with fast red (Sigma) as a substrate. Coverslips or slides were mounted in Moviol (Hoechst AG, Frankfurt, Germany). For double-immunofluorescence labelling, the two primary and two secondary antibodies were mixed and incubated simultaneously, and anti-goat IgG was rhodamine labelled.

All slides were examined on a Zeiss Axiophot microscope equipped with an incident fluorescence illuminator, using appropriate filters or, for alkaline phosphatase staining, using Nomarski optics. For evaluation and documentation of double labelling, an MRC 600 laser scan unit (Bio-Rad, Munich, Germany) attached to a Zeiss Axiovert fluorescence microscope was used, employing the CoMOS image analysis software (Bio-Rad). Micrographs were taken either in the Zeiss Axiophot by using Kodak Ektachrome 100 films or in the laser scan microscope by taking pictures from a high-resolution screen on Kodak TMAX 100 film.

Electron microscopy. For immunoelectron microscopy, cells were fixed with a mixture of 2% formaldehyde and 0.1% glutaraldehyde in PBS for 45 min. After being washed with PBS, cells were scraped off the culture flask, gently mixed with 2% warm, liquid agarose, and chilled at 4°C. Agarose containing suspended cells was cut into small pieces and then treated like small tissue blocks. These blocks were immersed overnight in 2.3 M sucrose containing 10% polyvinylpyrrolidone, frozen in liquid nitrogen, and cut into 100-nm sections with an ultramicrotome (Ultracut E; Reichert, Vienna, Austria) with FC4 cryoequipment as described by Tokuyasu et al. (7, 39). Sections were mounted on carbon-coated Formvar grids and after thawing washed with PBS. After treatment with 2% bovine serum albumin, grids were incubated with human serum diluted 1:100. After being rinsed in PBS, grids were incubated with goat anti-human IgG coupled to 10-nm gold particles (BioCell, Cardiff, United Kingdom). Finally, to embed and stain structures, the grids were floated, sections down, on 1.6% methylcellulose containing 0.2% uranyl acetate for 5 min. Excess methylcellulose was aspirated before air drying of the resulting thin film (11).

All electron microscopy preparations were examined in a Zeiss EM 902 electron microscope, and micrographs were taken on Kodak Estar electron microscope film, using the ESI mode.

Immunoblotting. For immunoblotting, 5×10^7 cells were lysed in 1 ml of Nonidet P-40 lysis buffer containing protease inhibitors phenylmethylsulfonyl fluoride (1 mM), sodium fluoride (10 mM), sodium pyrophosphate (1 mM), aprotinin (5 μg/ml), leupeptin (5 μg/ml), and sodium orthovanadate (1 mM) (all from Sigma). Lysates were separated on sodium dodecyl sulfate 10% polyacrylamide gels under reducing conditions and transferred to nitrocellulose membranes (Hybond Extra; Amersham, Braunschweig, Germany). The membranes were blocked with 5% nonfat milk in PBS and incubated with the appropriate serum diluted 1:500 (human sera) or 1:5,000 (goat anti-HERV-K Gag serum) in PBS containing 0.1% Tween 20. After extensive washing with PBS containing 0.1% Tween 20, filters were incubated with anti-human IgG or anti-goat IgG conjugated to horseradish peroxidase (Organon Teknika), which was subsequently detected by using an enhanced chemiluminescence kit (Amersham). Some membranes were reused repeatedly after removal of bound antibodies with 2% sodium dodecyl sulfate–100 mM mercaptoethanol in Tris-HCl at 56°C for 45 min.

For preadsorption experiments, antibody dilutions were incubated overnight at 4°C with either a lysate of *E. coli* BL21 (DE3) expressing recombinant HERV-K Gag protein or a lysate of wild-type BL21 (DE3), both diluted 1:25.

Nucleotide sequence accession number. The EMBL accession number for pcGU1 is Y08032.

RESULTS

Immunocytochemistry. We screened by immunocytochemistry on the HTDV-producing GH cell line 1,968 human sera derived from patients with germ cell tumors, from pregnant women, from groups of patients with various diseases, and from healthy blood donors. A reaction was considered positive when the labelling showed the same characteristic distribution as observed with the anti-HERV-K Gag positive control serum (Fig. 1). Positive sera also reacted with HTDV-producing Tera 1 cells but showed no labelling of cells that do not produce HTDV, such as T47D, PA-1, Tera 2, or MRC5 (not shown). A positive reaction against HTDV particles was confirmed by double immunofluorescence with goat anti-HERV-K Gag antiserum and the appropriate human serum. Localization of antigen was identical for both antisera; no labelling in addition to the typical Gag distribution was observed with human sera (Fig. 2). Specificity of the reaction could also be confirmed by immunoelectron microscopy: HTDV particles were specifically labelled in the same manner as with the goat anti-Gag serum. No additional structures were stained, indicating that only HTDV particles are recognized by the sera (Fig. 3).

Immunoblotting. To further characterize the immune reaction against HTDV, we performed immunoblotting experiments with cell lysates of GH cells. All sera that strongly reacted in immunofluorescence tests (i.e., sera of male germ cell tumor patients) and were tested by immunoblot reacted with the same two protein bands in GH cell lysates but not in lysates of the HTDV-negative Jurkat cells, a human T-cell leukemia line (Fig. 4). In contrast, sera of healthy blood donors negative in immunofluorescence did not react with these proteins (Fig. 4). The two proteins showed apparent molecular masses of about 80 and 90 kDa. The lower band represents the Gag precursor protein of HERV-K since it strongly reacts with the goat antiserum against HERV-K Gag. However, no processed Gag proteins could be detected in GH cell lysates, as maturation of Gag proceeds in retrovirus particles. When the sera were preabsorbed with lysate from recombinant *E. coli* expressing HERV-K Gag protein, the lower band with an apparent molecular mass 80 kDa completely disappeared, whereas the intensity of the upper band remained unaltered (Fig. 5). After preabsorption with lysate of wild-type *E. coli*, both bands were retained.

The immune reaction against these proteins could be observed only in the HTDV particle-producing cell lines GH and Tera 1, not in the nonproducing teratocarcinoma lines Tera 2 and PA-1 or in the human mammary carcinoma line T47D. In addition, cell lines infected with exogenous (SSAV) or endogenous (BaEV and MMTV) retroviruses were negative in immunofluorescence and immunoblot assays (Fig. 6).

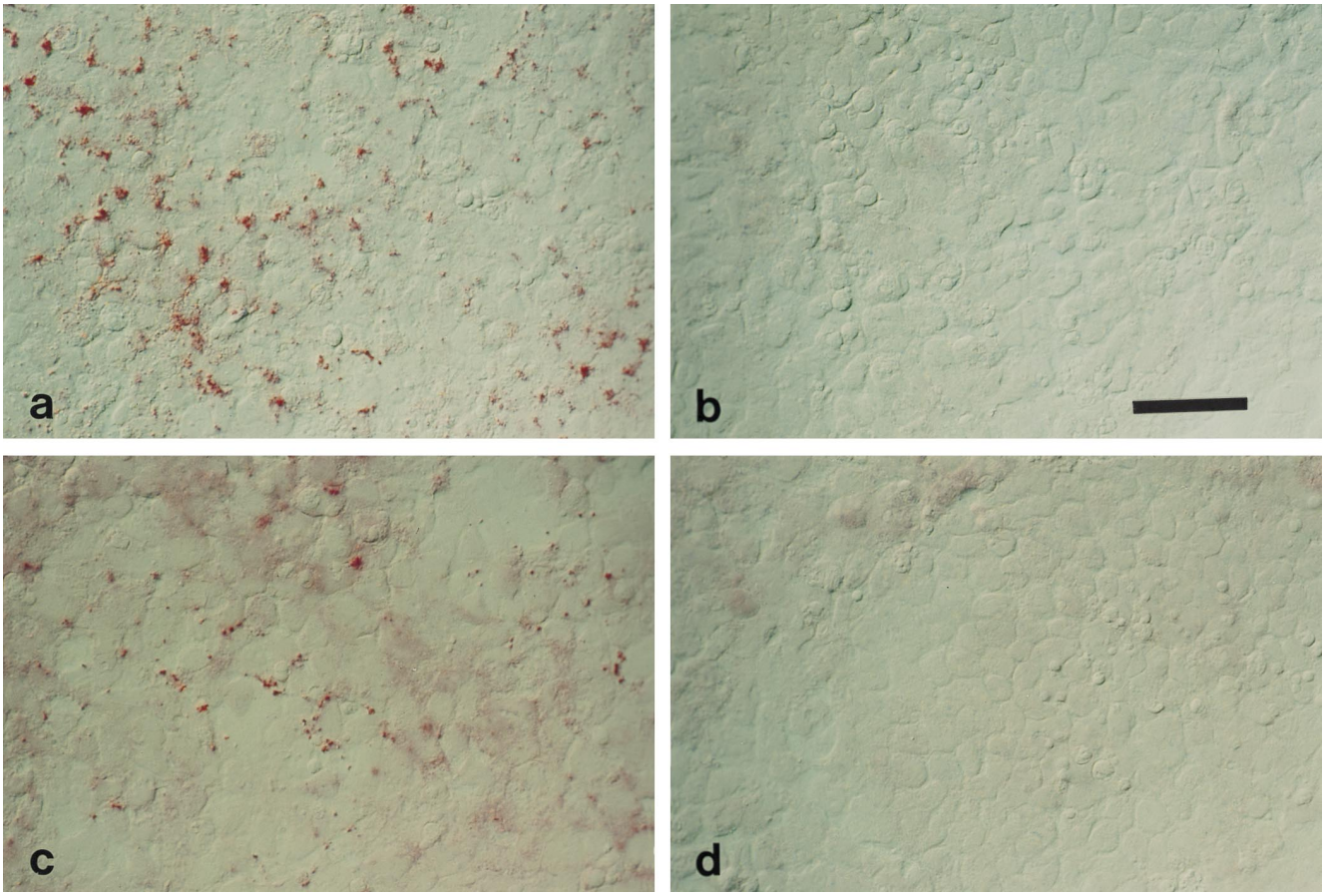

FIG. 1. Immunocytochemical staining of HTDV-producing GH cells with goat anti-Gag serum (a), corresponding preimmune serum (b), serum of a seminoma patient (c), and serum of a healthy blood donor (d). Second antibody was coupled to alkaline phosphatase and detected by using Sigma fast red reagent. Micrographs were taken by using Nomarski optics. HTDV Gag antigen is characteristically stained between adjacent cells where HTDV particles are budding. In panels a and c, staining occurs in the same pattern, although the human serum revealed slightly weaker labelling and higher background staining. The bar represents 50 μm.

Serology. During screening, sera were judged positive when they exhibited the typical staining pattern on HTDV-producing cells in immunofluorescence at a dilution 1:100. Among 1,968 sera tested, we found a total of 113 which showed such a labelling, with germ cell tumor patients showing the strongest reaction (Table 1). When titrated, the positive sera of testicular tumor patients had titers of 1:12,800, whereas the strongest sera from other groups of patients were positive only up to 1:800. Ten of 16 sera from germ cell tumor patients, collected at the time of tumor diagnosis, reacted strongly positive; 6 of the 10 were from patients with seminoma, and 4 were from patients with teratocarcinoma. The other six sera had no detectable antibody titer. From the nine sera of patients with germ cell tumors that were collected between 1 and 5 years after tumor operation, only one showed a slightly positive reaction. This serum originated from a teratocarcinoma patient 2.5 years after tumor removal. In one case of a teratocarcinoma patient, two consecutive sera were available; a strong reaction was observed only with the sample collected at the time of tumor diagnosis. In contrast, the serum was completely negative 5 years after tumor removal, when there were no signs of tumor recurrence (Fig. 4, lanes 4 and 5).

In addition to sera of germ line tumor patients, special attention was paid to sera of HIV-positive individuals and of pregnant women. Of 633 HIV-positive patients, 18 (2.8%) were positive for HTDV antibodies. This is in the range of the overall percentage of 3.9% of HTDV-positive sera in the healthy population. The anti-HTDV reaction of HIV-positive patients is most probably not due to a cross-reaction between HIV and HTDV, as only a minority of HIV-positive individuals were also positive for HTDV. Furthermore, in several cases, HTDV seroconversion was observed long after HIV seroconversion. In contrast to the successfully treated germ cell tumor patients described above, HIV-seropositive individuals never became negative for HTDV even after a period of several years. This was the case for all 16 patients from whom multiple serum specimens could be obtained over time. In four instances, anti-HTDV antibodies persisted continuously over a period of 8 years.

We also investigated the antibody status of pregnant women because retrovirus-like particles with morphology identical to that of HTDV were observed budding from syncytiotrophoblasts (14, 15). Of 393 sera, mainly from women in the ninth month of pregnancy, 32 were found to react with HTDV particles. The calculated incidence of 8.1% is significantly above the normal range of 3.9% ($P = 0.007$). The results obtained for umbilical cord sera, 10.6% of which were found to be positive, confirm these data.

A linkage between autoimmune disease and retrovirus expression has often been discussed (e.g., references 9 and 17). We therefore tested sera from patients with different autoimmune diseases for a potential reaction against HTDV. Despite

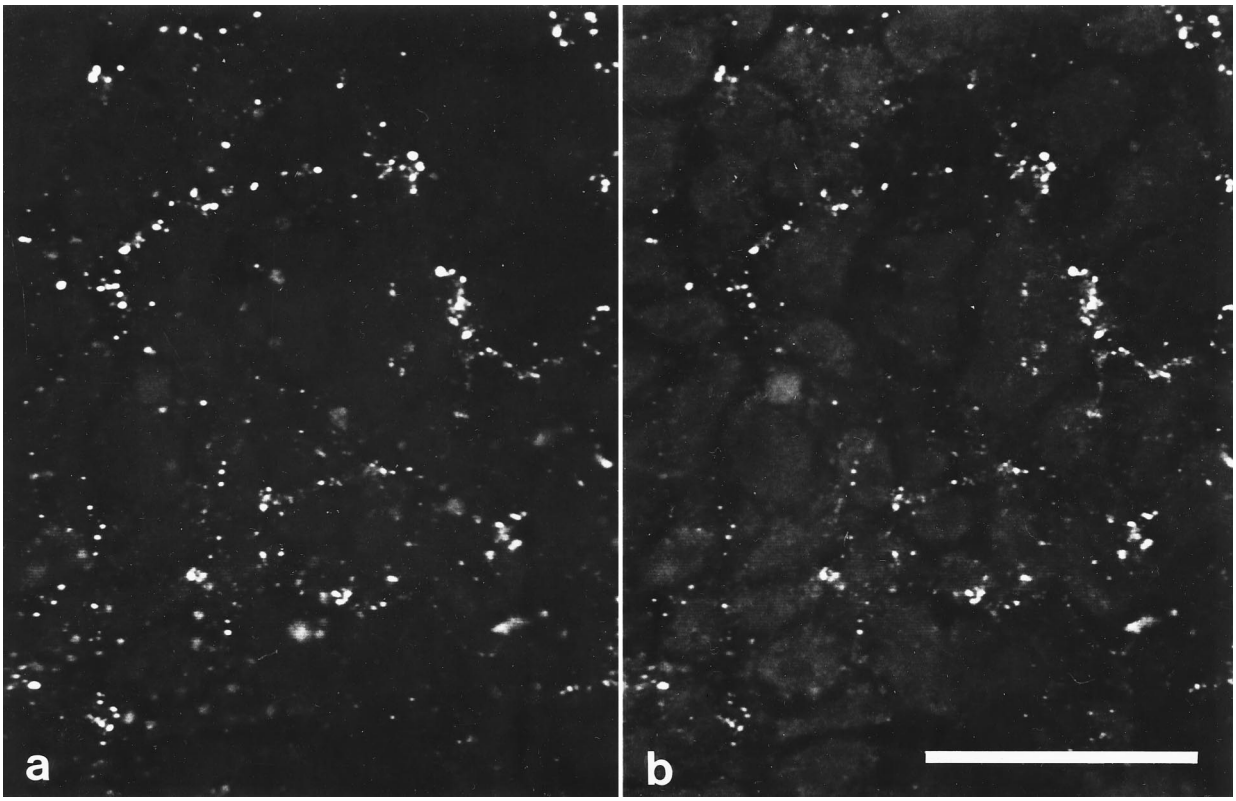

FIG. 2. Immunofluorescence double labelling of GH cells with goat anti-Gag serum (a) and a seminoma patient serum (b). Micrographs were taken with a confocal laser scan microscope. Both sera stain exactly the same structures around the cells. The bar represents 50 μm.

antinuclear and antimitochondrial antibodies, no reaction could be observed on GH cells in sera from 22 patients with rheumatoid arthritis. Two of 50 patients with multiple sclerosis were positive (4%), reflecting the percentage in normal blood donors. The remaining 28 HTDV-positive sera could not be linked to any disease status, as the vast majority of patients with comparable diseases were negative.

DISCUSSION

Using immunohistochemistry, immunoelectron microscopy, and immunoblotting approaches, we demonstrate that a specific antibody reaction against the human endogenous retrovirus HTDV/HERV-K exists in humans. Positive human sera clearly label HTDV particles in immunofluorescence and immunoelectron microscopy. In immunoblots of lysates of particle-producing cells, two bands with apparent molecular masses 80 and 90 kDa are detected by human sera. As shown with a specific goat anti-Gag antiserum and by preabsorption of human sera with recombinant Gag protein, the lower band represents the viral HERV-K Gag precursor. Interestingly, no cleaved Gag protein can be observed in these cell lysates. This result is in line with the morphological observation that mature HTDV particles with collapsed cores are rare and that the majority of virus particles remain arrested during budding at the cell membrane (1). It has also been shown for other retroviruses that particles with a defective viral protease exhibit an immature morphology characteristic of HTDV/HERV-K (10, 16, 46). However, Mueller-Lantzsch et al. found a functional protease in *E. coli* expressing recombinant HERV-K *gag* sequences (30, 36). It is therefore possible that the cloned HERV-K sequence is not identical to that expressed in teratocarcinoma cell lines, as it has been shown that several different HERV-K members are expressed in cultured teratocarcinoma cell lines (20, 23). Cleaved Gag proteins observed earlier in preparations of GH cell membranes (2) and in Tera 1 cell lysate and supernatant (35) might be explained by differences during preparation or by cellular or contaminating proteases.

All human sera that reacted with HTDV Gag protein in immunoblots also reacted with the 90-kDa band. Therapeutically treated germ cell tumor patients become negative for both proteins at the same time. Furthermore, in immunoblot analyses, both bands are always detected in equal intensity. All cell lines that were examined produced either both proteins (GH and Tera 1) or none (T47D, PA-1, Tera 2, MRC5, and Jurkat), suggesting that the 90-kDa band in the HTDV-producing lines and recognized by human sera is of viral origin.

No cross-reactivity exists between the 80-kDa Gag and the unidentified 90-kDa protein (Fig. 4 and 5). The higher-molecular-mass protein band may represent the viral Env protein, because in immunofluorescence and in immunoelectron microscopy, human HTDV-positive sera do not stain any other structures besides HTDV particles and because Env is the only major structural protein, other than Gag, of retroviruses. Calculated from sequence data, nonglycosylated Env protein of HERV-K has a theoretical molecular mass of 80 kDa (42). Since Env proteins of retroviruses are glycosylated, a molecular mass of about 90 kDa can be expected. However, transiently transfected COS7 cells and GH cells produced a recombinant HERV-K Env protein of 75 to 80 kDa (42), and the antibody used in that study did not react with the 90-kDa band recognized by human sera. Hence, direct proof that this protein indeed represents viral Env awaits the availability of

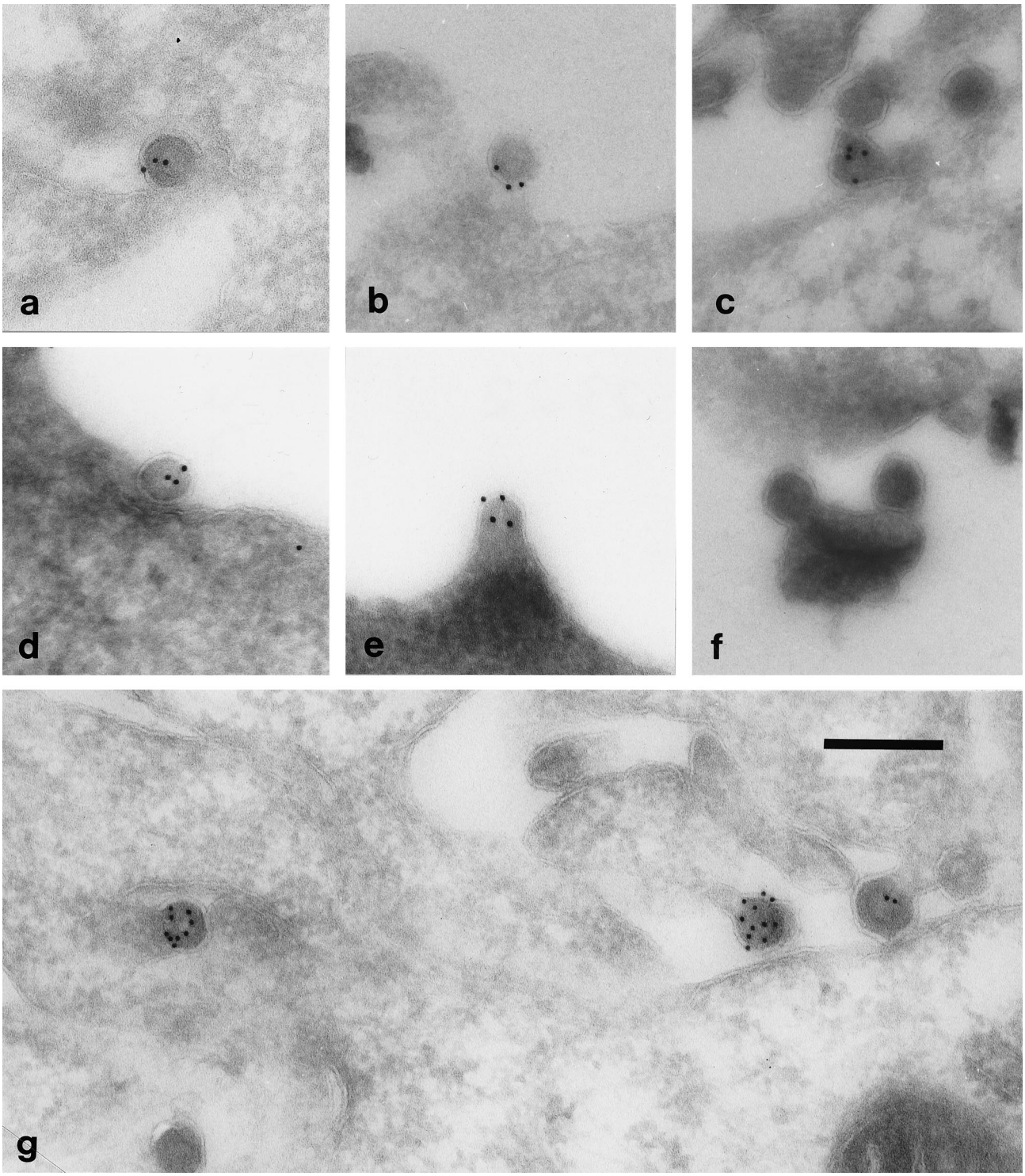

FIG. 3. Immunoelectron microscopic labelling of ultrathin frozen sections of GH cells with sera of germ cell tumor patients (a to e), with serum from a healthy person (f), and with goat anti-HERV-K Gag serum (g). Viral particles are specifically labelled with colloidal gold by the patient serum; no additional structures are stained. The bar represents 250 nm.

HERV-K-specific anti-Env antisera that recognize HTDV particles.

Cross-reactions between different retroviruses are common, and human immune reactions against animal retroviruses have repeatedly been reported (4, 18). However, the reaction to HTDV does not seem to be due to immune responses elicited by another retrovirus; only in cell lines known to produce HTDV particles could a specific reaction be observed, whereas cells infected with an endogenous or exogenous primate or mouse retrovirus were negative.

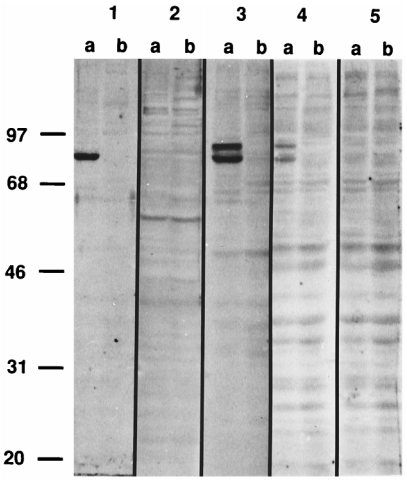

FIG. 4. Immunoblotting of HTDV-producing GH cell lysate (lanes a) and control Jurkat cell lysate (lanes b) with different sera: 1, goat anti-HERV-K Gag; 2, corresponding preimmune serum; 3, serum of seminoma patient 1336; 4, serum of teratocarcinoma patient 975 at the time of tumor diagnosis; and 5, serum of patient 975 5 years after tumor removal. While the goat anti-Gag antiserum recognizes only the 80-kDa HERV-K Gag precursor polyprotein, human sera stain an additional band of about 90-kDa of unknown nature. Sizes are indicated in kilodaltons on the left.

A main goal of our study was to try to correlate HTDV expression to disease. Indeed, correlation to disease is statistically highly significant in the case of male germ cell tumor patients, namely, patients with teratocarcinomas or seminomas. Of these patients, over 60% were HTDV seropositive at the time of tumor diagnosis. These patients also exhibited the strongest immune reaction against the virus. Antibody reactivity decreased rapidly after tumor removal. Since HTDV was initially found in a cell line derived from a human teratocarcinoma, it is reasonable to assume that HTDV is expressed as viral particles in those tumors. Our results are in full agreement with a recent report of Sauter et al. (35), who detected

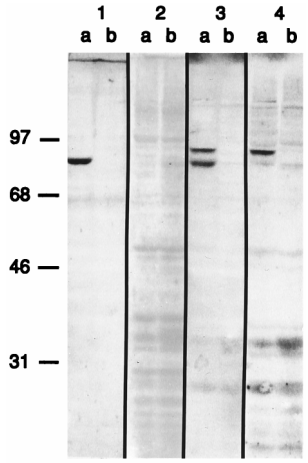

FIG. 5. Immunoblotting of HTDV-producing GH cell lysate (lanes a) and control Jurkat cell lysate (lanes b) with the following antisera: 1, goat anti-HERV-K Gag; 2, corresponding preimmune serum; 3, serum of seminoma patient 1336 absorbed with wild-type *E. coli* lysate; and 4, serum of patient 1336 absorbed with lysate of *E. coli* expressing HERV-K Gag protein. While the 80-kDa protein band disappears completely after preabsorption with Gag protein, the 90-kDa band remains unaltered. Sizes are indicated in kilodaltons on the left.

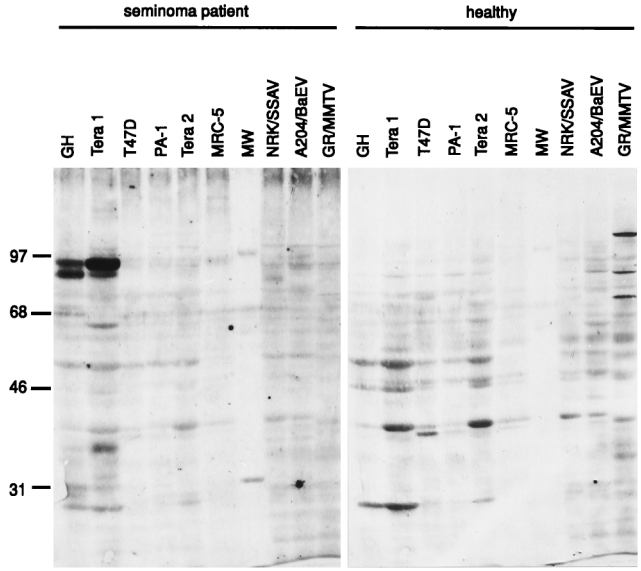

FIG. 6. Immunoblots of different cell lines stained with serum of a seminoma patient or a healthy person. The same blot was incubated first with the patient serum and then, after stripping of bound antibodies, with the control serum. Lanes MW represent molecular marker proteins. The background staining is similar for both sera except for the two HTDV-specific bands at 80 and 90 kDa in the HTDV-producing cell lines GH and Tera 1. No cross-reaction can be observed in cells expressing the exogenous simian retrovirus SSAV or the endogenous retroviruses BaEV and MMTV. Sizes are indicated in kilodaltons on the left.

antibody reactivity against HERV-K Gag protein at high frequency in sera from seminoma and teratocarcinoma patients. They also demonstrated proteins reacting with specific anti-HERV-K Gag antisera in the tumors. Even though we were not able to detect HTDV particles in germ cell tumors in situ, most likely for technical reasons, anti-HTDV reactivity might become a valuable tumor marker for diagnosis, prognosis, or therapy of testicular cancers. There is, however, no indication that HTDV itself might be the causative agent of germ cell tumors.

The findings reported here do not suggest an association of expressed HTDV/HERV-K with any disease other than germ cell tumors in male patients. Cohorts of patients with, e.g., autoimmune disorders did not react differently from healthy blood donors (Table 1). However, HTDV expression and the

TABLE 1. Antibody reactivities to HTDV particles in patients with different diseases and in healthy blood donors

Diagnosis	No. tested	No. positive	%	95% confidence limits
Germ cell tumor				
At time of tumor diagnosis	16	10	62.5	35.44–84.80
>1 yr after tumor removal	9	1	11.1	0.29–48.24
HIV positive	633	18	2.8	1.70–4.45
Pregnancy	393	32	8.1	5.63–11.27
Umbilical cord blood	198	21	10.6	6.69–15.75
Multiple sclerosis	50	2	4.0	0.49–13.71
Rheumatoid arthritis	23	0	0.0	0.00–14.81
Mammary carcinoma	4	0	0.0	0.00–60.23
Kaposi's sarcoma	5	0	0.0	0.00–52.18
Healthy	637	25	3.9	2.56–5.73
Σ	1,968	109		

induction of a corresponding immune response do not seem to be rare events in the normal population: about 4% of the apparently healthy population exhibit reactivity against HTDV. As the specific immune response against HTDV persists only for several months or years, as shown in this study for treated germ cell tumor patients, it can be concluded that most individuals express HTDV at some stage during their life times.

A function of endogenous retroviruses in the normal human placenta has been hypothesized by several authors (12, 31). Thus, our observation of slightly but statistically significant elevated antibody response to HTDV/HERV-K in pregnant women might be of interest. In conclusion, our data provide evidence that, as in the case of animal endogenous retroviruses, human endogenous retroviruses are expressed in vivo and that HERV expression elicits a specific immune response.

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