Specificity of Antibodies Directed against Env Protein of Human Endogenous Retroviruses in Patients with Germ Cell Tumors

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Abstract

We report here that 85% of the patients with germ cell tumors (GCTs) produce antibodies directed against Env protein of human endogenous retroviruses. Individuals that received antitumor treatment showed a decrease with time in their antibody titers. Importantly, of the rare cases of non-GCT individuals with Env-antibodies (n = 15, 0.8%), none produced antibodies directed against the transmembrane domain (TM), whereas all tested Env-positive GCT patients (n = 49) generated such antibodies at high titers. TM is required for Env to be expressed at the cell surface. Thus, anti-TM antibodies constitute highly specific markers for GCT and may hint at a function of Env during tumorigenesis.

Introduction

The human genome contains HERVs with structural and sequence similarities to infectious exogenous retroviruses (reviewed in Ref. 1). Most HERVs are defective because of multiple termination signals. One notable exception is the HERV-K10 provirus that was first identified by Ono (2); HERV-K10 clones contain few mutations that interrupt the putative coding regions. Recently, sequences harboring intact gag and env ORFs have been identified (3-5), and the viral protease ORF has been documented to encode a functional protein (6). Although HERV sequences seem to be transcribed in several human tissues, especially in the placenta and embryonic tissue, as well as in cell lines (for review, see Ref. 7), the corresponding proteins seem to be produced frequently only in GCTs (8). Accordingly, antibodies directed against HERV-K Gag proteins were found most frequently in patients with GCTs.

Materials and Methods

Cells. The insect cell line SF158 derived from Spodoptera frugiperda was maintained as described previously (9). Wild-type baculovirus AcNPV was amplified by infection of SF158 cells. Extracellular virions were prepared as described by Summers and Smith (10).

Eukaryotic Expression and Production of Polyclonal Antibodies. Genomic DNA was prepared from the peripheral blood lymphocytes of a healthy donor according to standard procedures (11). To develop the recombinant baculoviruses, insect SF158 cells were coinfected with BaculoGold™ (PharMingen, San Diego, CA) as a source of baculovirus DNA and recombinant transfer vectors carrying the fragments ENV1.9(+), ENV-OM, or ENV-TM (9).

For the purpose of raising polyclonal antisera, the env fragment (nucleotides 6794-7751) was expressed in the pATH vector system (12), and the emerged fusion protein was purified by electrophoresis in SDS-10% polyacrylamide gels and used to immunize rabbits as described previously (9, 5).

Immunofluorescence. For the determination of antibody titers against HERV-K Gag and Env, SF158 cells in logarithmic growth phase were infected with recombinant baculoviruses. Wild-type baculovirus-infected cells served as controls. Insect cells expressing HERV-K proteins were harvested 42 h postinfection and mixed with uninfected cells at a ratio of 1:40. Rabbit antiserum was used as positive controls to establish the system. Indirect immunofluorescence was carried out as defined elsewhere (8). To stain living cells, the method of Freeh et al. (9) was used.

Results

It is unclear whether the expression of HERV sequences has any relevance for the etiology of GCTs. However, considering that proviruses can encode polypeptides such as the p15E envelope proteins able to elicit immunosuppressive and anti-inflammatory responses (13), polypeptides expressed from endogenous retroviruses might very well be involved in the development or maintenance of the tumor. Therefore, it was interesting to ask whether HERV-K sequences can give rise to functional Env proteins and whether these can trigger an immune response in tumor patients and control individuals. In the first set of experiments, we aimed to identify HERV-K env sequences within the human genome that potentially encode functional proteins. For this purpose, DNA fragments covering env gene ORFs 5 and 6 were amplified by PCR from genomic DNA of human peripheral lymphocytes and subsequently subjected to sequence analysis. Fragment ENV1.9(+) encodes a putative protein from aa position 56-661 of the 699-aa full-length Env (4), lacking 38 aa of the COOH terminus. Expression of fragment ENV1.9(+) in SF158 insect cells via a baculovirus vector and subsequent Western blot analysis with a polyclonal antiserum directed against Env aa positions 116-435 resulted in the detection of a M, 80,000 glycosylated protein (Fig. 1A). Note that several signals of smaller-sized proteins were detectable in Lane 3 of Fig. 1A that most likely represent breakdown products of the M, 80,000 protein. Unfortunately, it has thus far proven difficult to express the Env protein in mammalian cells; therefore, the correct size of the glycosylated protein in these cells is still unknown. We then asked whether the truncated Env was correctly localized to the cell surface. To address this issue, we stained living SF158 cells that either did or did not express ENV1.9(+) with a polyclonal anti-Env antiserum. An anti-rabbit FITC-labeled antibody was used to detect primary antibody binding. Only the surfaces of cells that produced ENV1.9(+) stained positive, indicating that ENV1.9(+) is localized to the cell surface (Fig. 1B).

Antibodies directed against HERV-K Gag have recently been documented to be produced frequently only in patients with germ-line tumors (8). For this reason, and because of the potential of Env to support tumorigenesis, it was important to consider whether Env, like
HERV-K PROTEIN EXPRESSION AND ANTIBODY REACTION

Fig. I. A. immunoblot analysis of SF158 cells (1) infected with wild-type AcNPV (2) or recombinant baculovirus carrying the ENV1.9(+) fragment of HERV-K (3). The blots were probed with an anti-OM antiserum or preimmune serum as control. The apparent molecular masses were calculated from comigrating molecular weight standards and are given in kilodaltons. B. indirect immunofluorescence of living SF158 cells infected with recombinant baculovirus carrying the ENV1.9(+) fragment (a and c) or wild-type AcNPV (b). The cells were stained with either anti-OM antiserum (a and b) or preimmune serum as control (c).

Gag, is expressed preferentially in GCT and is able to induce a humoral response in patients with GCT. SF158 cells were either infected with baculovirus-expressing ENV1.9(+) or control infected with wild-type virus and were incubated with sera from different individuals. Again, antibody binding was detected through incubation with secondary FITC-labeled antihuman antibody and subsequent immunofluorescence analysis. Sera were screened at an initial concentration of 1:40 and in successive steps of 2-fold dilution. Table 1 summarizes the results obtained from healthy individuals and patients with various nontumor and tumor diseases. Of all screened sera in the control group of healthy individuals and patients with nontumor and tumor diseases. Of all screened sera in the control group of healthy individuals and patients with nontumor diseases, only 0.2 and 0.5% tested positive for the presence of anti-Gag and anti-Env antibodies, respectively. The titers ranged from 1:40 to 1:320. In the second control group comprising patients with non-germ cell or unspecified tumors, the fraction of Gag-positive and Env-positive sera was 1.9 and 1.0%, respectively. Antibody titers in this group were also in the range of 1:40 to 1:320. Note that the diagnosis "tumor diseases" quantifies patients with unspecified tumors and may thus contain some individuals with GCT.

Finally, patients with testicular diseases were examined (Table 1). Remarkably, whereas individuals in the different nontumor testicular diseases or non-GCT testicular tumors produced antibodies against Gag or Env only rarely (0 and 7%, respectively), patients with seminoma or mixed GCTs, generally referred to as GCT, produced these antibodies at very high frequency. Antibody titers in GCT patients were in the range of 1:640 to 1:10,240 or even higher. Anti-Env antibodies were found even more frequently (85%) than anti-Gag antibodies (up to 53%) in this group. The correlation of the presence of antibodies with GCT is further underscored by the fact that individuals with seminomas in remission as well as those with mixed GCTs in remission show a decrease in the fraction of seropositives. Likewise, individuals that received antitumor treatment showed a decrease with time in their titers against HERV-K Gag and HERV-K Env from a maximum of 1:5120 down to 1:40 or even undetectable levels (data not shown). We conclude that the production of anti-Gag antibodies and, to a higher degree, anti-Env antibodies is correlated with the presence of GCTs.

Although the presence of anti-HERV-K antibodies was most strongly correlated with GCTs, there were rare instances in which these sequences were expressed in non-GCT or nontumor diseases. We therefore asked whether it was possible to more specifically correlate GCTs with the presence of antibodies directed against defined epitopes on the Env protein. Instead of expressing ENV1.9(+) in SF158 cells, we now produced Env protein fragments from aa 56-465 (ENV-OM) or aa 463-699 (ENV-TM) via the baculovirus system and subsequently screened with patient sera as described above. Remarkably, all individuals with GCT and anti-Env antibodies tested thus far (n = 49) produced antibodies against an epitope on ENV-TM, in addition to antibodies against ENV-OM. Titers of the anti-ENV-TM antibodies were often three to four times higher than of anti-ENV-OM. In strong contrast, neither patients with non-GCT testicular tumors nor individuals with other diseases and positive for anti-Env (n = 15) produced antibodies that recognized the ENV-TM
epitope. Thus, GCT is strongly correlated with the expression of HERV-K, which is expressed in essentially all GCTs but rarely in other diseases. Anti-Gag antibodies and, more significantly, anti-Env antibodies are frequently found in patients suffering from GCTs (8). The strong correlation of HERV expression with GCT on the basis of the presence of HERV-K transcripts in several tissues and cell lines (1). However, assays in patients with breast cancer, HIV- and cytomegalovirus-infected individuals, or other diseases, albeit at a much lower frequency than in GCT tumors or control tissues. Thus, HERV expression and anti-HERV immune responses to HERV antigens have been observed in patients with other diseases, as well as healthy individuals. They obtained positive responses in 11.7-17% of the cases. This is a substantially higher frequency than the one we observed in comparable control groups. This discrepancy most likely results from differences in the experimental system. For instance, the detection of bacterially expressed Env by serum antibodies in the earlier study (15) suffers from higher background.

Certain infectious retroviral proteins as well as related polypeptides encoded by endogenous retroviruses exert immunosuppressive and anti-inflammatory functions. For instance, the Env p15E protein of different leukemia viruses is able to inhibit monocyte chemotactic response and lymphocyte blastogenesis in human cells in vitro (13). p15E has been shown to block IL-1-mediated signal transduction as well as the activity of protein kinase C (16, 17). Notably, p15E is identical to the TM of the Env protein. It is this region against which antibodies have been found to be directed specifically in patients with GCT but not in the rare cases of individuals with other diseases that were positive for Env antibodies. This points to the possibility that the TM region of HERV has a direct function in the maintenance or progression of GCTs through inhibition of an effective immune response. Because normal immune responses may be restorable in mice expressing p15E through the application of anti-p15E antisense oligonucleotides (18) or other means, this may point to new therapeutic strategies.

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**References**


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HERV-K PROTEIN EXPRESSION AND ANTIBODY REACTION


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