The full-length envelope of an HERV-H human endogenous retrovirus has immunosuppressive properties

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We have demonstrated previously that the envelope proteins of a murine retrovirus (MoMLV) and a simian retrovirus (Mason–Pfizer monkey virus) have immunosuppressive properties in vivo. This property was manifested by the ability of the proteins, when expressed by tumour cells normally rejected by engrafted mice, to allow the envelope-expressing cells to escape immune rejection and to proliferate. Here, it is shown that this property is not restricted to the envelope of infectious retroviruses, but is also shared by the envelope protein encoded by an endogenous retrovirus of humans belonging to the HERV-H family. These results emphasize the close relationship between endogenous and infectious retroviruses and might be important in relation to the process of tumour progression in humans.

The HERV-H family is a highly reiterated family of human endogenous retroviruses (reviewed in Löwer et al., 1996; Urvonovitz & Murphy, 1996; Wilkinson et al., 1994), most of which are defective elements carrying deletions, frameshifts and/or stop codons. However, a systematic search for HERV-H elements possessing an envelope gene with a large ORF led to the identification of a single provirus containing a complete envelope (GenBank accession no. AY289709; Lindeskog et al., 1999; de Parseval et al., 2001). As illustrated in Fig. 1(a, b), this env gene is part of a provirus located on chromosome 2, at position 2q24.3. In vitro translation of the cloned gene reveals a 62 kDa protein (Fig. 1(d)), as expected for the identified ORF, which also contains hydrophobic domains, probably corresponding to the envelope fusion peptide and membrane anchorage domains (Fig. 1(a)). Interestingly, a domain with a sequence closely related to a previously identified immunosuppressive domain of the murine retrovirus Moloney murine leukaemia virus (MoMLV) can be identified, at a homologous position (Fig. 1(a); see Cianiolo et al., 1985). We therefore tested the immunosuppressive activity of this endogenous envelope by using the procedure that we devised previously (Mangeney & Heidmann, 1998), which is illustrated in Fig. 1(c). The complete env gene was first inserted into an expression vector that also encoded a hygromycin-resistance gene under the translational control of an internal ribosomal entry site (IRES). The previously described murine tumour cells MCA205 and CL8.1 (Tanaka et al., 1988; Mangeney & Heidmann, 1998) were then transduced with these vectors and hygromycin-resistant cell populations were isolated. These cells expressed the stably transduced envelope vector, as demonstrated by coupled RT–PCR and in vitro translation assay (results for MCA205 cells are shown in Fig. 1(d); similar results were obtained for transduced CL8.1 cells, but are not shown). The transduced cells were finally injected subcutaneously into immunocompetent mice, under conditions of either allogeneic or syngeneic engraftment, and the occurrence of tumours and tumour sizes were then determined twice or three times a week.

In a first series of experiments, we used methylcholanthrene-induced murine fibrosarcoma MCA205 tumour cells (H-2b haplotype) in an allogeneic context. As illustrated in Fig. 2(a, bottom), when injected into an allogeneic host (five to ten BALB/c mice per group; H-2b haplotype), MCA205 control tumour cells did not lead to tumour formation in the engrafted animals. Under the same conditions, MCA205 cells expressing the HERV-H envelope were able to form easily detectable tumours that persisted for at least 2 weeks in a large fraction of the engrafted animals (Fig. 2(a, top)). This enhancement of tumour cell growth was not observed, under identical experimental conditions, with ‘irrelevant’ expression vectors encoding transmembrane proteins unrelated to retrovirus envelopes (the murine CD2 and erythropoietin receptor proteins; data not shown and Mangeney & Heidmann, 1998). Induction of tumour formation was not due to any difference in intrinsic growth rates between the control and HERV-H envelope-transduced cells, as tumour development induced by...
the two cell populations was identical when engrafted into a syngeneic host (C57BL/6, H-2b haplotype), (Fig. 2a, insets).

In a second series of experiments, we used the previously characterized CL8.1 tumour cells (H-2b haplotype), which can be engrafted under syngeneic conditions, thus mimicking more closely a physiological process of spontaneous tumorigenesis, and can still be rejected by the mouse immune system. Injection of these tumour cells into a syngeneic host (five to ten C57BL/6 mice per group; H-2b haplotype) indeed led to the formation of small tumours in only a limited fraction of the engrafted animals (0–20%; Fig. 2b, bottom), although they grew into large tumours in all cases when the mice were rendered immunodeficient by prior X-ray irradiation (see below). With HERV-H envelope-transduced CL8.1 cells, we observed that expression of this envelope severely enhanced tumour growth in mice (Fig. 2b, top): a large fraction of the immunocompetent mice (up to 100%) developed tumours that grew continuously, leading to animal death. Again, tumour growth was not due to intrinsic differences in rates of proliferation between the envelope-transduced and control cells, as similar profiles were observed for the two cell types when engrafted into X-irradiated hosts (Fig. 2b, insets). Clearly, expression of the HERV-H envelope gene, as demonstrated previously for the Mason–Pfizer monkey virus and MoMLV envelopes (Mangeney & Heidmann, 1998; Blaise et al., 2001), enables the transduced tumour cells to escape immune rejection in immunocompetent mice in both an allogeneic and a syngeneic context.

These results therefore provide the first demonstration that an endogenous retrovirus possesses an envelope with immunosuppressive properties in vivo, and thus they extend further the already recognized similarities between infectious and en-
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dogenous retroviruses. They also provide a hint for a possible role of these elements in vivo, and especially in the development of tumours in humans. Being induced in several tumour cells lines and tissues (reviewed in Löwer et al., 1996; Urnovitz & Murphy, 1996; Wilkinson et al., 1994), endogenous retroviruses could participate in tumour progression via the expression of an immunosuppressive env gene product. Several approaches could possibly help in assessing this role. Firstly, an extensive analysis of HERV-H envelope expression in both normal and tumorous human tissues, after raising appropriate antibodies for immunohistochemical analysis, might provide evidence for a correlation between expression of the immunosuppressive protein and tumour expansion. Secondly, despite the high copy number of HERV elements in the human genome, only a few gene copies are expected to encode a full-length envelope protein (Lindeskog et al., 1999; Tönjes et al., 1999; Voisset et al., 2000; de Parseval et al., 2001). Accordingly, the complex genetic problem of the assignment of a definite function to highly reiterated gene families might be reduced to a more ‘classical’ single-copy gene characterization, amenable to genetic approaches: searching for polymorphisms within the presently identified env gene copy among the human population (as performed for the ERV-3 locus in de Parseval & Heidmann, 1998) or for an association between the identified locus and a susceptibility locus for the formation of tumours might provide hints for a role of the identified gene locus in a human disease.

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References
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