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Endogenous retroviruses and multiple sclerosis

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Endogenous retroviruses are normal constituents in vertebrate genomes. They have been associated with various diseases of presumed autoimmune etiology. However, conclusive evidence of their significance as susceptibility factors in these diseases is still lacking. In our laboratory we have focused attention upon endogenous retroviruses as candidate genes in multiple sclerosis. In this communication we describe general properties of endogenous retroviruses and we present observations from some of our studies. *Journal of NeuroVirology* (2000) **6,** S80–S84.

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General properties of endogenous retroviruses

Large numbers of endogenous retroviruses are present as normal constituents in genomes of perhaps all eukaryotes (Wilkinson *et al*, 1994). As opposed to exogenous retroviruses, which are infectious agents with a horizontal mode of transmission, the endogenous counterparts are mendelian elements transmitted vertically (genetically). Retroviruses have a genomic structure with a long terminal repeat (LTR) at both ends, containing elements for initiation and modulation of transcription in addition to other regulatory elements. In between there are at least three major genes, designated *gag*, *pol* and *env*, encoding for internal structural proteins, reverse transcriptase and envelope glycoprotein, respectively. Most of the endogenous retroviruses in the human genome are related to exogenous animal retroviruses. Only a few human endogenous retroviruses with partial homologies to the exogenous human retroviruses, such as the human T cell lymphotropic viruses (HTLVs), have been reported thus far (Wilkinson *et al*, 1994). Many endogenous human retroviruses are present in multiple copies per haploid genome but a few single-copy elements have been identified, including ERV3 and the HTLV-related endogenous sequence, HRES-1.

Most likely, endogenous retroviruses are the footprints of ancient retroviral infections which have spread to germ line cells or early embryos and integrated into the chromosomes of these cells. In this way an infectious retrovirus has become a permanent resident of the genome. Subsequent reintegration of the 'endogenised' retrovirus would lead to the appearance of many copies of it within the genome. Most likely, many of these integrated viruses would be detrimental to the host and as a consequence of this the majority of them have accumulated stop codons and thereby lost their infectious capacity during evolution. However, some retroviral genes still have intact reading frames. Perhaps these reading frames encode proteins which are useful to the host.

Endogenous retroviruses are often categorised as mobile elements. That is, they are able to transpose through a mechanism called retrotransposition, mediated by reverse transcriptase. Also other elements which encode for reverse transcriptase and resemble endogenous retroviruses but lack the LTRs are able to retrotranspose, i.e. the so-called non-LTR retrotransposable elements. Insertion of such an element into a gene may cause inactivation of this gene. Fortunately, retrotransposition rarely takes place.

Many endogenous human retroviruses are transcriptionally active. There is also evidence that some endogenous human retroviruses express proteins. Of interest, endogenous retroviruses in various animals, including mice (Coffin, 1984), chicken (Coffin, 1984), pigs (Wilson *et al*, 1998), baboons and their close relatives (Todaro *et al*, 1976) are able to produce particles. Similarly, a few human endogenous retroviruses seem to have a potential for production of particles. However, this

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is probably not a general phenomenon since most endogenous human retroviruses have stop codons in their reading frames or are truncated, precluding expression of full-length proteins. Apparently, expression of endogenous retroviral particles in humans takes place under certain conditions – perhaps during the course of autoimmune diseases such as MS. Whether this is essential to the pathogenesis of these diseases or an epiphenomenon is not known. Another question of interest is whether particles produced by human endogenous retroviruses are infectious.

Possible functions and significance of endogenous retroviruses

Still the functions of endogenous retroviruses are obscure. Most likely, different endogenous retroviruses carry out different functions and some may not even have a function at all. So far, there is reason to believe that endogenous retroviral elements participate in the transcriptional regulation of genes in their vicinity. A previous study even suggested that a retrovirus promoter had replaced the normal promoter of a human gene, namely HLA-DRB6 (Mayer *et al*, 1993).

Also the gene products of endogenous retroviruses could have important functions. Findings that many endogenous retroviruses are abundantly expressed in the placenta combined with an ability of some retroviral gene products to induce cell fusion have led to speculations that they participate in the formation of placenta. Even more interesting, incorporation of retroviruses into the germ line of primitive pre-mammal species has been postulated to have initiated the evolution of placental animals (Harris, 1991).

There is evidence to suggest that endogenous retroviruses take part in the immune regulation and perhaps contribute to the development of autoimmune diseases (Table 1). Several exogenous retroviruses produce a transmembrane protein with various immunosuppressive effects, including inhibition of the production of proinflammatory cytokines (Haraguchi *et al*, 1997). For example the murine leukaemia viruses produce p15E transmembrane proteins with such effects. Some endogenous retroviruses encode similar or related factors, suggesting that the host has learned to exploit the incorporated retroviral genes for its own purposes during evolution. Perhaps the p15E-related factors are of importance as immunological down-regulators in autoimmune diseases.

Table 1 Immune effects of endogenous retroviruses

(1) Effects due to integration sites.
(2) Effects due to gene products:
  (a) immune regulatory functions – p15E and P15E-related factors, murine superantigens, human superantigens?
  (b) acting as autoantigens.
(3) Interactions with exogenous retroviruses.

A question which has been addressed by several studies concerns the possible role of endogenous retroviral gene products as autoantigens. Indeed, elevated immune reactivities against endogenous retroviral components have been detected in several autoimmune diseases (Banki *et al*, 1992; Li *et al*, 1996; Hishikawa *et al*, 1997). However, the interpretation of these results are often complicated by a significant degree of individual variation in the responses.

Murine self-superantigens are encoded by an open reading frame in the 3′ LTR of mouse mammary tumour viruses (MMTV) (Simpson *et al*, 1993). They are expressed on the surface of B cells and bind to particular Vβ chains in the T cell receptor. Binding of superantigens to T cells takes place during ontogenesis and results in the deletion of T cell subsets. This may influence the susceptibility to various experimental autoimmune diseases, probably reflecting a preferential Vβ usage of the T cells responsible for induction of these diseases. Whether there exists human endogenous superantigens is a question of considerable interest. If the human genome encodes factors with superantigen activity they would be expected to possess relatively weak activities since complete deletions of human T cell Vβ subsets do not appear to be common. Alternatively, such putative factors could have activities somewhat different from their murine counterparts.

Endogenous retroviruses are capable of interacting with exogenous retroviruses though a variety of different mechanisms, including recombination and phenotypic mixing. By recombinational events with endogenous counterparts an exogenous retrovirus may acquire nonneutralisable epitopes and perhaps escape the immune surveillance of the host (Rasmussen, 1997 and references therein).

Human endogenous retrovirus ERV3

The full-length endogenous human retroviral segment ERV3 is transcribed in leucocytes, adrenal glands and several types of cancers (Andersson *et al*, 1998). The *env* gene of this endogenous retrovirus encodes a polyprotein with several glycosylation sites (Cohen *et al*, 1985). Processing of this polyprotein results in the formation of a typical surface unit and a transmembrane protein lacking the hydrophobic region. The latter of these two proteins contains a stretch related to the immunosuppressive motif of the p15E transmembrane proteins of murine leukaemia viruses. This has led to speculations that ERV3 participate in the immune regulation. Another interesting finding is

that readthrough transcripts composed of ERV3 and a downstream zinc finger-like sequence are expressed in some types of cells (Kato *et al*, 1990). Previously, these composite transcripts were believed to encode a transcriptional protein with tumour suppressing activity, but this idea has now been abandoned (Boyd *et al*, 1993).

Of interest, a genetic association between ERV3 and rheumatoid arthritis has been reported based upon restriction fragment length analysis (Rubin *et al*, 1991). Using the same approach a subsequent study was not able to confirm this finding (Takeuchi *et al*, 1995). More recently, ERV3 has been associated with autoimmunisation during pregnancy and congenital heart block, a disease in babies probably caused by transplacental passage of maternal autoantibodies (Li *et al*, 1996). We have conducted a study to identify polymorphisms in the *env* and the distal portion of the *pol* gene of ERV3. This study revealed a surprisingly large number of single nucleotide substitutions (Rasmussen and Clausen, 1998a). One of these substitutions changed an arginine codon to a stop codon in the *env* gene (Table 2). The allele with the stop codon encodes a truncated version of the *env* polyprotein without the transmembrane protein. We decided to compare the frequencies of this allele in MS patients and healthy individuals since the transmembrane protein of ERV3 could be a down-regulator of immune responses. Our examination revealed similar and low frequencies of the allele with the stop codon in MS patients and healthy control subjects. Of interest, a few individuals homozygous for the stop codon were detected. So far, the possible consequences of having the allele with the stop codon on both chromosomes are unknown. In another study of ERV3 and MS we stratified for HLA-DR2 (unpublished data). We found that the frequency of the ERV3 allele with the stop codon did not differ significantly between DR2-positive MS patients and healthy control subjects carrying this HLA determinant. Similarly, the frequency of the stop codon-carrying allele was practically identical in DR2+ and DR2− negative patients. None of these findings associated ERV3 with MS.

Table 2 Genetic variation in the *env* gene of ERV3

Position[a]	*Nucleotide/amino acid substitution*
149 (*env*, SU)	T (ile)→C (thr)
455 (*env*, SU)	G (cys)→A (tyr)
547 (*env*, SU)	C (arg)→T (stop)
587 (*env*, SU)	A (tyr)→G (cys)
1322 (*env*, TM)	G (ser)→A (asn)
1586 (*env*, TM)	G (ser)→A (asn)
1629 (*env*, TM)	T (val)→C (val)
1727 (*env*, TM)	T (leu)→C (ser)
1859 (*env*, TM)	G (arg)→A (gln)

[a]Nucleotides are numbered starting with the probable ATG initiation codon. SU and TM are abbreviations for envelope surface unit and transmembrane protein, respectively. Modified from a recent study (Rasmussen and Clausen, 1998a).

Human endogenous retrovirus HRES-1

The human T cell lymphotropic virus (HTLV)-related endogenous sequence, HRES-1, is a truncated retroviral segment (Perl *et al*, 1989), which is expressed in various cell types including leucocytes (Rasmussen *et al*, 1995). Previously, antibodies against a gene product of HRES-1 were detected in MS and other autoimmune diseases (Banki *et al*, 1992). On the basis of three single nucleotide substitutions we have defined four haplotypes of this endogenous retrovirus, numbered 1–4. Of interest, HRES-1 haplotype 2 and 3 appear to be associated with MS in Denmark (Rasmussen and Clausen, 1999). To address the question whether this association reflected linkage disequilibrium with a susceptibility allele on a neighbour locus or a causal relationship between MS and HRES-1, we examined individuals of another ethnic origin, namely Shanghai Chinese (Rasmussen *et al*, 1998b). Unfortunately, we were not able to answer this question since an association between MS and HRES-1 was not detected in the Shanghai Chinese population.

In a subsequent study we examined distributions of HRES-1 in MS patients and control subjects from the United Kingdom (UK). We found that haplotype 1 appeared at increased frequency in patients as compared with controls (Rasmussen *et al*, submitted). This was in contrast to the findings from Denmark. Since the haplotypic distributions of healthy control subjects were comparable in the UK and Denmark a possible interpretation of our findings is that genetically different subsets of MS associated with different HRES-1 alleles exist in the two countries. On stratification for DR2 we did not find a statistical significant difference in HRES-1 haplotype distribution between patients and control subjects from the UK.

Examination of individuals from France did not reveal a difference in the distribution of HRES-1 haplotypes between MS patients and healthy control subjects (unpublished results). However, HRES-1 haplotype 4 was more frequent in DR2-negative than DR2-positive patients. The detection of associations between MS and different HRES-1 haplotypes in different countries is both surprising and interesting. They raise the question of HRES-1 being a marker of genetically distinct subsets of MS. Moreover, the association between MS and HRES-1 could be more or less restricted to DR2-negative subjects.

Human endogenous retrovirus family HERV-K

The family of endogenous human retroviruses designated HERV-K is related to MMTV (Ono *et*

al, 1986). Like many other endogenous retroviruses HERV-K is present in multiple copies, i.e. about 50 per haploid genome. The HERV-K family is believed to have integrated into an Old World primate species about 30 million years ago. Of interest, several of the members of this family still have open reading frames.

Evidence has been presented that HERV-K encodes an endogenous superantigen and that the development of insulin-dependent diabetes mellitus (IDDM) is associated with such a factor stimulating Vβ7-carrying T cells, including those with an autoreactive potential (Conrad *et al*, 1997). A mechanism similar to that have been proposed for induction of autoimmune diseases by exogenous superantigens, e.g. some bacterial toxins. Disappointingly, subsequent studies failed to confirm the initial findings of an association between IDDM and HERV-K (e.g. Murphy *et al*, 1998).

We have searched for differences in the sequence of HERV-K segments between MS patients and healthy control subjects. To accomplish this, members of this endogenous retroviral family were amplified. Subsequently, the amplified fragments were inserted into plasmid vectors. After transformation, HERV-K segments now present in bacterial clones were analysed. Using this approach we found that a large proportion of HERV-K segments from the same individual differed in sequence. There also appeared to be a significant degree of variation in the sequence of HERV-K segments between different individuals. Still, the data are too sparse to assess whether MS patients differ from healthy individuals.

Concluding remarks

Many aspects with respect to function and significance of endogenous retroviruses are uncertain. Furthermore, the investigation of some retroviruses is complicated by their high copy number. Whether endogenous retroviruses are implicated in autoimmune diseases is unknown at present. So far, our studies have revealed an association between MS and the endogenous single-copy retroviral element HRES-1. Further studies have been initiated to confirm this.

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