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Human Endogenous Retroviruses: Nature, Occurrence, and Clinical Implications in Human Disease

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INTRODUCTION

AIDS has directed attention to the possible pathologic effects that human retroviruses and their endogenous analogs (human endogenous retroviruses [HERVs]) may have on several organ systems. Extensive studies during the last decade on the molecular biology of human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1) have provided both the intellectual framework and the molecular methodology to test whether, and under what circumstances, HERVs may be implicated in the pathogenesis of a spectrum of debilitating diseases. Some associations are well known: B-cell immunoglobulinopathies (1); inflammatory diseases of the nervous system (160); autoimmune rheumatic and connective tissue diseases (94, 159, 180, 191); and chronic fatigue immune dysfunction syndrome (40). Others have been accorded little recognition even though type C retrovirus particles occur rather abundantly in tissues, i.e., in human reproductive tissues (82, 100) and psoriasis lesions (78, 79). The aim of this review is to collate and appraise the data bearing on the question of whether HERVs can be implicated in specific disease entities. We address a number of basic questions: what is the nature of HERVs detected to date; what factors appear to regulate their expression; what are the possible pathogenic

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consequences; and what clinical diagnostic tests can be used to detect HERVs or their gene products.

CHARACTERISTICS OF RETROVIRUSES

Retroviruses are diverse and found in all vertebrates examined to date. There is also a spectrum of retroviruslike transposable genetic elements (56) that have been found in plants, fungi, and eukaryotic protists and in prokaryotes that use reverse transcription to reproduce their RNA sequences. Depending on their genetic constitution, such retroelements are called retrotransposons or retroposons (76). They are of interest because they provide an insight into the origin and evolution of retroviruses and into retrovirus gene sequences that we recognize as HERVs. The only feature that regularly distinguishes retrotransposons from genuine retroviruses is the absence of an envelope. Genetically simpler retroelements, in decreasing order of gene structure (56, 201), are retroposons, retrons, and retrosequences. Generically, they have been called transposons. They are relevant to consider here because they direct our attention to the orders of complexity we may find among HERVs and to evolutionary considerations. Doolittle et al. (44) published a fascinating paper on the possible origins and evolution of retroviruses and retroviruslike genetic elements. These authors suggest three evolutionary schemes: "the gene clusters that constitute the background of prototype retroviruses could have been gathered together as long as the time of the prokaryotic-eukaryotic diversion; or retrovirus-like entities in the form of mobile elements or viruses, or both, have existed in parallel with host cells from the very beginning of cellular life forms; and infectious retroviruses may have originated relatively recently in vertebrate animals—perhaps within the last hundred million years and after the emergence of birds and mammals, and are now spreading through the biological world by horizontal infection." We subscribe to Temin's view (183) concerning retroviruses; viz., they are mobile genetic elements packaged into an envelope that facilitates entry into other cells. More explicitly, the retrovirus genome is replicated by a flow of genetic information through a DNA intermediary, a process called reverse transcription. The responsible RNA-dependent DNA polymerase (reverse transcriptase [RT] or *pol*) makes up part of the virion and becomes active early after viral attachment-penetration. The DNA intermediary is covalently integrated into host cell DNA and becomes virtually indistinguishable from "normal" host cell DNA sequences. This capability confers a distinctive feature to retroviruses. They may incorporate sequences in their genomes that confer oncogenicity (oncogenes) or host-derived oncogenic sequences (proto-oncogenes). We favor the idea (183) that retrovirus sequences can play an important role in turning on or off, or amplifying, gene sequences needed (119) for tissue differentiation during embryogenesis.

The biological characteristics of major retrovirus groups are summarized here so that the occurrence of HERVs in human cells and tissues can be put into perspective. Bernhard (12) and Dalton (38) categorized retroviruses into morphologic types A, B, C, and D. Dalton emphasized that thin-section electron microscopy preparations were fraught with artifacts; for example, only budding type C particles can be identified with certainty in tissues or cells, and a specific identification of type B particles in milk or tissues is very difficult. So-called intracisternal type A particles (IAPs; see below) may be found in cisternae or associated with the endoplasmic reticulum in the cytoplasm.

TABLE 1. Contemporary simple and complex retroviruses

Class	Properties	Prototype(s)
Simple	Type C, avian	Avian leukosis, sarcoma, and reticuloendotheliosis viruses
	Type C, mouse	Mouse leukemia, sarcoma viruses
	Type C, cat	Feline leukemia, sarcoma viruses
	Type B, mouse	MMTV
	Type C, simian	Gibbon ape leukemia virus
	Type D, simian	Mason-Pfizer monkey virus
Complex	Lentivirus, sheep	Visna virus of sheep
	Lentivirus, goat	Caprine encephalitis-arthritis viruses
	Lentivirus, horse	Equine infectious anemia virus
	Lentivirus, human	HIV-1, HIV-2
	Nonlentivirus, human	HTLV-1, HTLV-2

Intracisternal Type A Particles (IAPs)

IAPs were of limited interest to most retrovirologists until recently. A surge of interest has been elicited by the findings of Garry et al. (57), who detected IAPs in a human T-cell lymphoblastoid line after exposure of the cells to salivary gland extracts obtained from patients with Sjögrens syndrome. The literature on IAPs is complex. Kuff and Lueders (96) have published a detailed and precise review of the molecular biology of IAPs.

As a group (57, 96), IAPs have a number of common biological properties. Their dark "rigid" annular structure, without an indication of an envelope or a budding process, is fairly easy to identify by thin-section electron microscopy. They are common in some transplantable mouse tumors, particularly the myelomas. They are far more common in Syrian than in Chinese hamsters. They are not released from cells and do not have an extracellular phase. They cannot be transmitted by injection to any host tested to date or by cell cocultivation or cell fusion. In their more complete form, they have a genome analogous to that of retrotransposons except they have an envelope (*env*) gene defect(s). The *env* region contains multiple stops in all reading frames. These stops are highly conserved among different IAP entities (96). The *env* defect(s) could explain their inability to carry out a complete replicative cycle.

The molecular characteristics of IAPs are complex but have been classified (96) into distinct type 1 elements that make up the major class of IAP sequences and consist of 7.1-kb elements. Four subclasses that represent deletions of the basic type element are defined. Type 2 IAP elements have a 0.5-kb insertion absent from type 1 elements. They, too, have deletions that serve as a basis for dividing them into three subclasses. The biologic effects of these IAP classes have not been well characterized. It is doubtful (96) that they play a role in early phases of embryogenesis. However, transpositions of IAP elements have been observed in IAP-rich mouse tumor cell lines. Such transpositions (96) can have a specific effect on target cell gene expression that can be either increased or decreased. Examples include the κ light chain, interleukin-3 (IL-3), and the c-*mos* oncogene. The complex nature of IAPs means that comparative studies of HERVs and IAPs will be far more complex than the literature indicates.

Retrovirus Classification

It is possible (37, 110) to group retroviruses into two broad categories on the basis of the complexity of their genomes and

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mechanisms of replication, viz., simple or complex retroviruses. For "simple" retroviruses (Table 1), replication involves three virus-encoded genes (*gag*, *pol*, and *env*). The integrated provirus encodes two classes of transcripts: genomic RNA (which codes for *gag*, *pol*, and *env* as explained below) and spliced RNAs that code for viral enzymes or structural components. For the purposes of this review, we found it most helpful to list (Table 1) the common simple retroviruses according to their primary natural hosts. By contrast to the simple retroviruses, the lentiviruses have a complex genome, up to 10 kb in size, that codes for the *gag*, *pol*, and *env* gene products and regulatory genes as well. Such regulatory genes are transcribed early in the replicative cycle. They regulate how much genomic and spliced mRNA is made and how much is transported to the cytoplasm for translation. These steps are affected in a major way according to the physiologic status of the host cell. For example, unstimulated $CD4^+$ T cells ordinarily are not productively infected by HIV-1. We will discuss below the importance of cell factors in the regulation of exogenous retroviral expression and, by implication, the possible regulation of HERV expression.

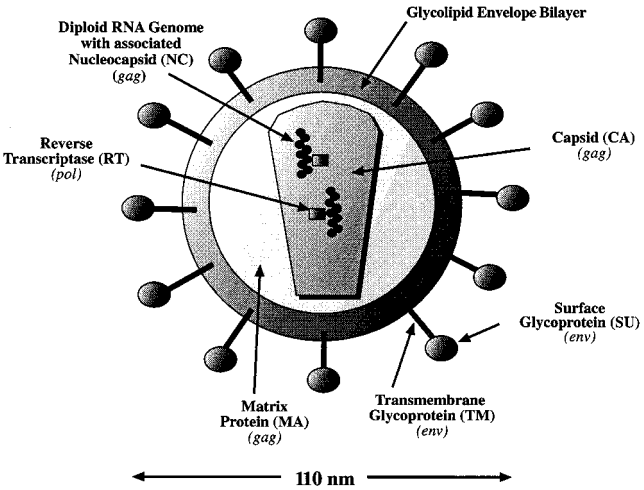

FIG. 1. Schematic diagram of a representative exogenous retrovirus. *pol* represents the *pol* gene that has RT and RNase functions. The envelope lipid bilayer and associated spikes contain the *env* glycoproteins that make up heterodimeric complexes of the transmembrane and surface domains. The capsid, nucleocapsid, and matrix proteins are cleavage products of *gag* gene polypeptides.

Avian Retrovirus Model

The avian retroviruses comprise four natural groups (36, 149, 194), based primarily on the diseases they induce, and subgroups based on their envelope glycoprotein antigens, interference patterns, serum neutralization patterns, and avian host-range infectivity studies. Most breeds of chicken carry and transmit avian leukosis-sarcoma viruses horizontally. Congenital transmission occurs from infection by virus that is excreted from the oviduct into the albumin and thence to the embryo. Genetic transmission occurs by means of viral genomes incorporated into the germ line. Somatic and germ line cells of common commercial chickens carry either complete or replication-defective (see below) viral genomes. Commonly, each chicken cell may carry 5 to 30 integrated viral genes termed endogenous viral loci. Some have the capacity to become exogenous and transmissible. The diversity of such endogenous viruses and lack of a phylogenetic relationship suggest that such endogenous viruses arose in chickens after speciation but before domestication. The genetics of susceptibility are complex and cannot be reviewed here. Susceptibility depends on the virus subgroup and strain, dose effects, and genetic constitution of the host.

With the development of primary cell culture techniques (184) it became possible to analyze virus-cell genome interactions. For example, when low doses of the Bryan high-titer strain of Rous sarcoma virus (RSV) were used to transform primary chicken embryo cells, such transformed cells failed to produce detectable quantities of virus unless exposed to superinfection by an unrelated avian virus. When this was done, the cells produced significant amounts of RSV-like particles (46). Ultimately, it was shown (195, 196) that the original RSV nonproducer (NP) cells carried a defective endogenous virus that required superinfection by "helper" virus to permit a full cycle of endogenous virus replication. Endogenously produced virus (RSV-0), defective in replication, was detected in NP cells (165, 197) by DNA hybridization techniques. In some cases, infectious virus was inducible (197) from NP cells by chemical or physical inducing agents.

The described results are relevant in attempts to detect HERVs: they document that a defective endogenous retrovirus genome can be expressed if missing gene functions are provided by a suitable superinfecting helper virus, i.e., a phenomenon known as complementation; that a defective endogenous virus can be induced by a physical agent such as X-irradiation or chemical agents such as the halogenated pyrimidines; that the induced endogenous virus may form pseudotypes with the helper virus to yield progeny with a new, albeit temporary, host range; and that the events described may be only temporary or spasmodic. However, if such events occur in vivo, they may suffice to elicit antibodies to viral agents that otherwise might go undetected.

Retrovirus Replication

To appreciate the circumstances under which HERVs may be expressed, and how expression may be regulated, one must understand retrovirus replicative mechanisms in considerable detail. The scheme that follows takes this into account. Since the published literature clearly shows that host cell factors can play a significant role in HERV expression and regulation and can be of equivalent importance in the pathogenesis of disease, we begin development of this theme here. As appropriate, we identify the importance of host cell factors in the replication of retroviruses. Our presentation draws considerably on the excellent review of Luciw and Leung (110). Leis et al. (103) recently published a standardized and simplified nomenclature for proteins common to all retroviruses.

The structure of a representative simple retrovirus is shown in Fig. 1, and the corresponding genome is shown in Fig. 2. Attachment of the virion to the cell is mediated by a fusion of the viral envelope (*env* glycoprotein) with a virus-specific receptor on the cell membrane. Receptors ordinarily determine retrovirus cell and tissue specificity, and host range as well. When contiguous cells fuse, a syncytium results. They are found commonly in foci of fibroblasts transformed by RSV.

Retrovirus replication is divisible into the early and late phases. The former involves attachment and penetration with the consequent release of the core into the cell cytoplasm. The viral RT and the diploid RNA viral genome make up a functional cytoplasmic nucleoprotein complex. DNA transcripts are detectable as early as 2 h after infection and are transported to the nucleus, where they are integrated randomly into the host cell genome. In the mouse model for ecotropic viruses, as discussed below, products of the $Fv\text{-}1^b$ allele appear to

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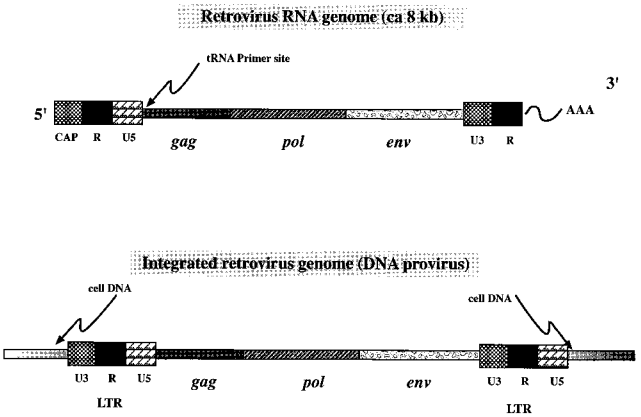

FIG. 2. (Top) Diagram of a simple retrovirus genome showing the 5′ cap, the 3′ poly(A)tail, and the tRNA primer site. U3 and U5 are unique sequences located where shown. When the retrovirus genome is reverse transcribed (bottom), the U3 and U5 sequences are duplicated as LTRs that contain the principal regulatory sequences. Viral RNA transcription is initiated from the provirus genome at the U3–R boundary. The CCAAT and TAATA start signals and enhancer-promoter sequences are located upstream from the RNA initiation-transcription site and specifically bind cellular transcription factors that are explicit proteins often of nucleolar origin (Fig. 3).

inhibit the integration step (84, 106) to result in nonproductive infection. This phenomenon appears to differ from that found when quiescent T cells are infected nonproductively by HIV-1. In the latter case (206), reverse transcription of HIV-1 DNA is incomplete. When such cells are exposed to mitogenic stimulation, productive infection ensues. Zack et al. (206) suggest that mitogenic stimulation leads to the activation of cellular or viral processes, which allows the completion of reverse transcription.

In the late phase of replication, viral genomic DNA has been integrated into host cell DNA and is transcribed in the nucleus by host RNA polymerase II complexed with host factors that can have a major effect on the efficiency of RNA initiation and transcription. Two classes of transcription products are generated, viz., unspliced genomic RNA for incorporation into mature virions and spliced RNA transcripts (subgenomic RNA) used to generate retrovirus polyproteins. Translation of retroviral proteins takes place on cytoplasmic polysomes. Posttranslational processing (proteolytic cleavage) is mediated by either viral or host cell proteases. The major regulatory events occur at the level of transcription and involve *cis*-acting elements of the viral DNA genome.

Since the long terminal repeats (LTRs) of the integrated viral DNA are of paramount importance in initiation and transcription, their molecular organization must be considered. A specific host cell tRNA is bound near the 5′ end of the viral genome that is required to prime DNA synthesis. Each group of related viruses employs a specific tRNA primer. During reverse transcription, the RT has two primary functions. One RT domain functions primarily as the reverse transcriptase. The other has RNase H activity; i.e., it degrades the RNA moiety of the intermediate RNA-DNA hybrids. The double-stranded DNA copy of genomic RNA is longer than the original template because of the duplication of direct repeat sequences at each end, viz., the LTRs. The mechanism of genomic DNA integration into the host cell DNA is unusual in that double-stranded linear DNA is the form of the molecule integrated. In the process, the integrase sequence presumably holds ends of the linear DNA in close proximity and causes staggered cuts at the ends of the LTRs and in cell DNA. After integration, each LTR has lost two base-pair units. There are six base-pair repeats of cellular DNA flanking the insertion. As noted above, retrovirus gene insertion can cause insertional mutations that may activate oncogenes (24), inactive tumor suppressor genes (129), have a lethal effect (64), or alter coat color (34). It is not clear whether HERVs may act similarly, but the possibility is not excluded.

Cell tropism and tissue specificity for retroviruses have been redefined at the molecular level; i.e., cell and tissue tropism need not be exclusively a matter of cell surface receptor specificity for virus. Instead, the dynamics are the interactive events between cell factors, usually nuclear or nucleolar in origin, and viral regulatory sequences in the LTRs. This is an important conceptual advance. This advance appears not to have been exploited adequately in combination therapeutic approaches in AIDS or autoimmune or neurologic diseases that have a multifactorial etiology. For HERVs, such dynamics appear to be of particular importance because they involve the regulation and expression of HERV gene sequences and host responses to expressed HERV gene products. Therefore, it is necessary to examine rather closely how transcription is initiated and regulated for the simple retroviruses. This lays the foundation for analyzing the regulation of HERV expression.

In brief (Fig. 3), the 5′ LTR has promoter activity that initiates viral RNA synthesis. It has binding sites for cellular proteins important in the initiation and regulation of transcription. Both cellular and viral promoters have a TATA box with a sequence that binds cellular factor TBP (TATA-binding protein). These moieties make up the cellular transcription apparatus involving cellular RNA polymerase II. All retroviruses studied to date have a TATA box in the U3 domain. Elements upstream from the TATA box specifically bind cellular transcription factors. The TATA box, along with these upstream elements, is referred to as the basal promoter. As one might expect, the U3 domain of some retroviruses has negative regulatory elements that impair or prevent the initiation of retrovirus RNA synthesis.

As noted previously, enhancers are *cis*-acting sequences that regulate (enhance) viral and cellular promoters. It is important to note that enhancer elements may function in only certain tissues, or in specific cell types, or only during some stages of the cell cycle, or in some animal species and not others. Their occurrence in tissues and cells, or their absence, helps to explain retrovirus tissue or cell tropism independent of cell surface receptors for virus. Enhancers also possess nucleotide sequences (core elements) that specifically bind host cell factors. Such factors are proteins (core-binding factors) that recognize both viral and cellular enhancers. Moreover, transcriptional enhancers of several retroviruses act only in explicit cell types.

Simple retroviruses, unlike the lentiviruses, do not encode specific proteins to regulate the transcription of RNA destined to be incorporated into virions or for the synthesis of structural or enzymatic proteins. Instead, transcription is regulated primarily at the level of the LTRs and by *cis*-acting sequences that function as splicing controls. Such splicing events dictate how much genomic RNA is going to be encapsidated into virions or spliced into subgenomic transcripts that will be employed for the synthesis of viral enzymatic or structural units. Polyproteins generated from such subgenomic transcripts are cleaved by either viral or host cell proteases.

The mechanism of virion assembly is relevant since an explanation is needed for the common finding that full-length HERV transcripts, or their translation products, are found in human cells yet viral particles appear not to be produced, occur with very low frequency, and lack infectivity. Helpful

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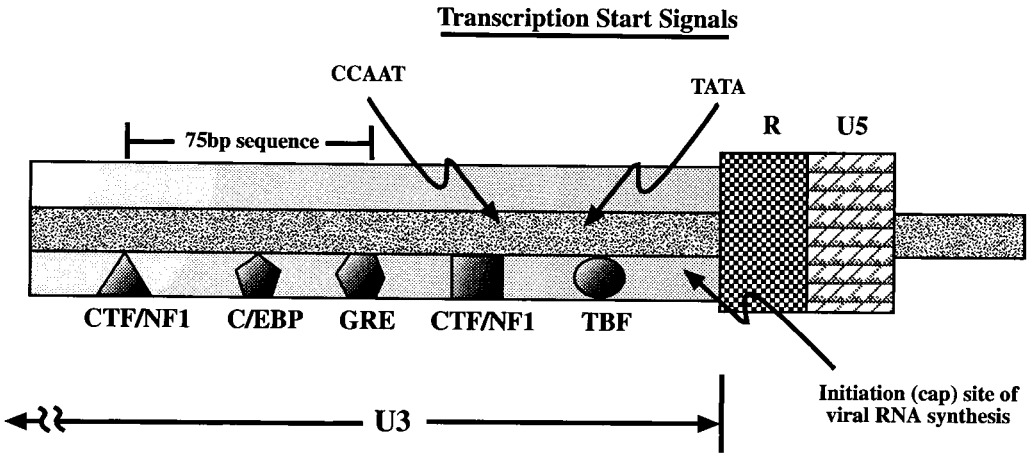

FIG. 3. U3 of a 5′ LTR of a simple retrovirus LTR expanded (not to scale) to indicate transcriptional control binding sites for cellular transcription factors: TBF, TATA binding factor; CTF/NFI, CCAAT transcription factor/nuclear factor 1; GRE, glucocorticoid response element of MMTV (found in both the left and the right LTRs of MMTV). When complexed with RNA polymerase II, TBF and CTF/NFI factors can regulate the initiation of elongation of viral RNA synthesis. Core/enhancer binding protein (C/EBP) and glucocorticoid response elements tend to be tissue and/or cell type specific in response to glucocorticoid hormones and hence are important in MMTV and some families of HERV expression.

guidelines may be derived from the assembly process of simple retroviruses in which the assembly process seems to be regulated in a number of interesting ways. The packaging of viral subunits into an infectious entity is not an efficient process. One factor that increases the probability of success is the focal synthesis of precursors in the cell; i.e., the proximity of components facilitates assembly. Second, the conventional retrovirus genome bears *cis* packing signals (designated Ψ) that distinguish viral genomic RNA from host cell RNA. Such *cis*-acting signals appear to interact with the virion core proteins important to the encapsidation process. There are three packaging signals (recognition elements) that, when functional, increase (45) the efficiency of the packaging of viral genomic RNA ca. 1,000-fold. Although the details remain unclear, it is established that *gag* is required for capsid (core) formation. The *gag*-derived nucleocapsid protein associates closely with genomic RNA during core assembly. It appears to have two functions (123): dimer formation by genomic RNA and the annealing of tRNA primer to viral RNA. There is controversy concerning the assembly-budding process involved in the generation of mature retrovirus virions. Nevertheless, for type C particles, it is reasonably clear that budding through the cellular plasma membrane is facilitated by the matrix proteins. There is also a consensus that such virions acquire *env* glycoproteins in their envelope by budding through the portions of the plasma membrane rich in such glycoproteins.

Mouse Leukemia-Sarcoma Model

The data from the avian and mouse retrovirus models reveal a diversity of molecular composition and biological activity. Thus, it is reasonable to expect an occurrence of HERVs ranging from those that are replication competent and inducible to those that are as short as LTRs. Similarly, one might expect to find HERV genomes with internal deletions in structural genes or in LTR regulatory elements, or with gene sequence rearrangements or inserted host gene sequences, etc. One also would expect to find host-cell or tissue specificities that are based on specific host-cell cofactor requirements.

Table 2 defines the key properties of infective and defective endogenous mouse and avian retroviruses. Only ecotropic and xenotropic viruses (Table 3) occur naturally integrated into the mouse germ line. Both are found in laboratory and feral mice. It has been estimated that there are, on average (36, 92, 194), 10 to 50 endogenous viral genomes per haploid cell. The key questions for these viruses are as follows: what are the genetics of their inheritance and expression; what other factors determine their expression; are they inducible; how do endogenous retroviruses interact with each other, with exogenous retroviruses, and with host cell nucleotide sequences; and do they behave in a way in mice that is informative for understanding HERVs. Guidelines to these questions are available as noted below.

By using inbred mice with a high copy number of integrated ecotropic virus in their germ line and by backcrossing them with appropriate low-frequency strains, genes determining endogenous retrovirus transmission and expression have been delineated with NP cells (196) that express virus spontaneously (albeit at low frequency) or after induction (197). It was documented that infectious ecotropic virus production was determined by Mendelian genes. As noted above, the expression of these ecotropic viruses is permissive in $Fv\text{-}1^{n/n}$ mice (106) but not in mice that carry an $Fv\text{-}1^{b}$ allele. An analogous mechanism may be operative in the disparate occurrence of tropical spastic paraparesis in closely related human populations infected by HTLV-1 (81). A subclassification (65) of ecotropic viruses into N, B, and NB types provided a basis for making distinctions within a closely related group. Perhaps more pertinent to HERVs, some proviral genes were expressed only as viral proteins. This phenomenon will be noted again below for the xenotropic viruses.

TABLE 2. Categories of endogenous proviruses in mouse and chicken cells

Class	Genome structure and properties
Infective	Complete provirus exists in cells, sometimes is released spontaneously; is readily inducible by X-irradiation, halogenated pyrimidines, cell mitogens, or cytokines
Defective	Genome is complete but defective in one or more gene functions; may be rescued by superinfecting helper virus, complementary virus, or host cell or tissue cofactors

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TABLE 3. Terminology for mouse leukemia-sarcoma viruses

Term	Property
Ecotropic	Only infects cells of the natural host (mice)
Xenotropic	Will not infect cells of the natural host
Amphotropic	Infects cells of both natural and foreign hosts (also called dual tropic or polytropic)
N tropic	Subclass of ecotropic viruses that replicate best in NIH Swiss mice
B tropic	Subclass of ecotropic viruses that replicate best in BALB/c mice

Xenotropic provirus sequences occur in all laboratory strains of mice and in feral mice as well. Many are inducible as described above. The NZB strain of mice produces large amounts of xenotropic virus in vivo and in vitro. It has been used (186) as a model for immune complex diseases simulating systemic lupus erythematosus (SLE). In the NZB strain, virus production is controlled by unlinked loci. For example, Gxx and Xen CSA play a major role in regulating the production of the gp70 glycoproteins important in immune complex formation and in the pathogenesis of the SLE-like syndrome in the NZB strain.

Mouse Mammary Tumor Virus (MMTV) Model

Type C retroviruses have been found repeatedly in the reproductive tissues of humans and subhuman primates, yet we know little about their possible biologic effects. One striking feature is their apparent hormonal responsiveness. Since the hormonal responsiveness of retrovirus-induced mammary carcinoma in mice is well established, it provides a relevant model. We discuss hormone responsiveness here in some detail because many of the reported HERVs are hormone responsive. In brief, developmental and physiologic processes in metazoans are regulated by a relatively small number of cholesterol derivatives, e.g., the steroid hormones. Their regulatory functions reflect (205) a highly conserved evolutionary history. Their mode of action, according to central dogma (162), involves first the binding of a hormone to a soluble cytoplasmic receptor protein. As a result of binding, the receptor becomes "activated." The activated steroid-receptor complex is transported to the nucleus, where it stimulates the transcription of target genes to generate mRNAs that are cell or tissue specific. Their translation into new proteins represents and defines the hormonal response of a cell or tissue.

As a result of evolutionary constraints, all hormonal receptors have a similar structure (10) consisting of three domains: a variable N-terminal region; a short and highly conserved cysteine-rich central domain; and an equally conserved C-terminal domain. The central domain is the DNA binding moiety. A segment proximal to the C-terminal domain is important for intranuclear localization. Steroid receptors make up two groups: glucocorticoid, progesterone, androgen, and mineralocorticoid receptors; and estrogen, thyroid, retinoic acid, and vitamin D_3 receptors. Steroid receptors complexed with their hormonal ligands bind to specific cellular DNA sequences to increase the efficiency (205) of transcription initiation from nearby promoters. There is a consensus (155) that the interaction between transcription factors and hormone-receptor complexes may stabilize the transcription complex. Topological features of the chromatin can affect significantly hormone-receptor binding to DNA and, consequently, the level of hormonal stimulation. The hormone response elements of MMTV are not tightly restricted (10) and are responsive to glucocorticoids, progestins, androgens, and mineralocorticoids. Glucocorticoid responsiveness is not an exclusive property of MMTV since glucocorticoid responsiveness has been reported (26) for some mouse leukemia viruses.

MMTV is distinct from other simple retroviruses in important respects. In thin-section electron microscopy, the MMTV viral particle is relatively electron lucent and has a rather fragile-looking envelope with rather long, hairlike projections typical of type B retroviruses. The genome of 9 kb is somewhat larger than that of most simple retroviruses and contains a 3′ open reading frame (ORF) that encodes a 36-kDa protein showing (2, 13) many similarities to minor lymphocyte-stimulating antigens. The encoded minor lymphocyte-stimulating antigen functions as a superantigen and has been designated the superantigen gene product (SUG). Finally, the MMTV encodes (162) its own hormone regulatory region in its left and right LTRs. In infected cells, however, the left LTR provides the major promoter function. The glucocorticoid regulatory region maps 140 to 190 residues upstream from the start of transcription. This region encodes several copies of glucocorticoid response elements, i.e., DNA sequences that bind the glucocorticoid receptor.

Whether MMTV has specific integration sites is unclear. Ringold (162) argues that integration is random. The data reviewed by Kozak and Ruscetti (92, 93) suggest otherwise. Notwithstanding, Fan (49) points out that after insertion the MMTV LTR activates several proto-oncogenes whose products are similar to fibroblast growth factor. Not only do they cause abnormal growth, but they also act as paracrines; i.e., they stimulate the growth of contiguous cells. One is led to wonder whether the hormonally responsive type C virus-producing cells in human reproductive tissues will display some of these characteristics.

HIV-1 AND HTLV-1 MODELS

Much of the contemporary research on HIV-1 and HTLV-1 (37, 58, 61, 110, 111, 136, 147, 148, 153, 176) now has focused on the mechanisms that regulate their gene expression. This has led to an appreciation of how the gene functions that normally regulate lymphocyte and tissue-specific gene inducibility and expression (6, 35, 117) can affect HIV-1 and HTLV-1 replication and expression. The following principle (117) has emerged: specific host cell factors interact with both cellular and viral promoters and enhancers to either positively or negatively affect cell and retrovirus gene expression. There are, in addition, transport factors (27) that direct such host cell factors to the nucleus (or nucleolus), where they bind to specific host cell DNA sequences or their gene products. Host cell factors may function either *cis* or *trans*, may or may not be position or orientation specific, and may or may not affect contiguous cells. The last point may have particular relevance to Kaposi's sarcoma (47). To grasp the dynamics, it is essential to have a general understanding of how HIV-1 replicates and how HIV-1 gene expression is regulated.

HIV-1 Replication

As a group, the lentiviruses are complex. They have at least eight genes that encode regulatory proteins in addition to the *gag-pol-env* genes of the simple retroviruses. They tend to infect cells nonproductively, appear to replicate best in cells of the macrophage lineage, are pantropic in their cellular host range, and are distinctive in their dependence on host cell factors for productive virus infection. HIV-1 is typical in all of these respects.

The HIV-1 genome (Fig. 4) is ca. 9.5 kb and is made up of

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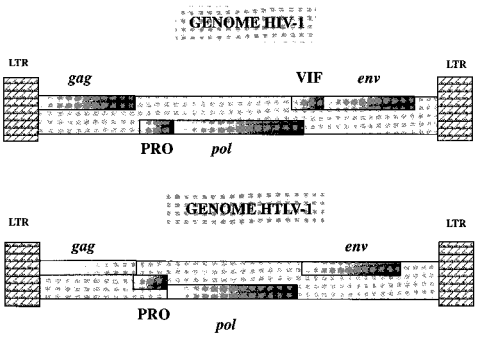

FIG. 4. HIV-1 and HTLV-1, because of their genetic complexity, are longer (ca. 9.5 kb) than simple retroviruses (ca. 8.0 kb). In addition to *gag*, *pol*, and *env*, they contain gene sequences that code for regulatory proteins that determine how much viral genomic RNA (unspliced) and spliced RNAs are made during the early and late stages of viral synthesis. For reasons of simplicity, the major regulatory genes of HIV-1 (*tat* and *rev*) and HTLV-1 (*rex* and *tat*) are not depicted. PRO represents the sequences that code for the corresponding viral proteases.

two broad categories of genes. *gag*, *pol*, and *env* code for viral constituents or enzymes. The remaining genes are regulatory. These, in turn, make up two categories, i.e., either essential or accessory. *tat* and *rev* serve as master switches that dictate the kinds and amounts of RNA (spliced or unspliced) transcribed from the DNA of integrated provirus during the early and late phases of replication. Both are required for replication. The accessory genes (111) seem to function as fine-tuning agents of the replicative process. Although they may have a dampening effect, more often they are enhancive.

The envelope spikes of HIV-1 are made up principally of glycoproteins gp120 and gp41. For T cells, particularly those that have been activated, gp120 binds to the T-cell $CD4^+$ receptor prior to internalization of the viral core. The HIV-1 genome is released into the cell cytoplasm as an RNA-protein complex; from there it is reverse transcribed to its cDNA structure prior to transport to the nucleus, where it is integrated into the host cell DNA apparently without specific integration sites. There are some peculiarities of the cytoplasmic process of reverse transcription. Thus, Zack et al. (206) reported that quiescent (resting) T cells do not fully convert genomic RNA into the corresponding full-length DNA complement by reverse transcription. Analogous findings have been made for both the avian and mouse retroviruses. Paradoxically, in such abortively infected T cells, normal viral replication ensues if such cells are subjected to subsequent mitogenic stimulation. Several inferences can be drawn from these observations. First, such abortive T-cell infections are not restricted to the lentiviruses. Second, host cell cofactors, possibly of exogenous origin, may facilitate productive cellular infection. Third, cofactors of extracellular origin could play an activating role in the dynamics of tissue infection. Again, these phenomena appear not to have been exploited in combination therapeutic approaches in AIDS or in autoimmune or neurologic diseases that have a multifactorial etiology.

The early phase of HIV-1 replication is characterized by a basal level of RNA transcription. The early class of viral transcripts consists predominantly of doubly spliced, ~2-kb mRNAs that encode the *trans*-acting regulatory proteins Tat and Rev. In the absence of Tat, the level of transcriptional initiation directed by the viral LTR is low. Transactivation occurs when the *tat* gene product binds to TAR (a virus-coded, 59-nucleotide RNA) in conjunction with the cobinding of cellular proteins that are sequence specific for the TAR moiety of the Tat-TAR complex. The result is the formation of a transcriptional complex with RNA polymerase II that increases the efficiency of transcription initiation-elongation approximately 100-fold. During the early phase of viral replication, Tat levels are relatively low and the transcription of doubly spliced ~2-kb mRNAs predominates over that of unspliced 4.0- to 9.0-kb RNAs. The early spliced RNAs code for regulatory proteins. Among these is the key regulatory nuclear phosphoprotein Rev. Rev binds to a viral gene-coded RNA called RRE (Rev responsive element). Parslow (147) recently published an excellent review on Rev functions. When Rev reaches sufficient concentration, it directs the selective transport to the cytoplasm of spliced and genomic RNAs needed for viral assembly and maturation. In the words of Luciw and Shacklett (111), it functions as a molecular "chaperone" causing a switch from the early to late phases of viral replication.

The polyprotein precursors translated from viral mRNAs by cytoplasmic ribosomes are cleaved into their final product by either viral or host cell proteolytic enzymes. The immune response to them may be of value for diagnostic application and of predictive value in the prognosis of disease, or they may mimic host tissue antigens and thus elicit autoimmune responses, etc. We discuss clinical applications and significance below.

Interaction of Host Cell Factors with LTRs

One theme of this review is that exogenous cofactors may significantly affect the replication or expression of HIV in vivo. Similar phenomena may affect HERV expression. Thus, it is important to learn how exogenous cofactors may impinge on HIV replication and gene expression. The most important regulatory viral gene sequences reside in the LTRs, particularly the promoters and enhancers. The LTRs contain the binding sites for host cell factors (often of nuclear or nucleolar origin) that effect promoter-enhancer activity. Other host cell factors bind to viral gene-specified RNAs, as noted for Tat and TAR above, and increase transcriptional efficiency or rate. Maniatis et al. (117) pointed out that host cell factors that regulate gene expression in T-cell activation or that act as developmental regulators (6) can act on the gene sequences that regulate HIV replication or gene expression. Consequently, exogenous factors that elicit a mitogenic response, or superantigens, phorbol esters, coinfecting viruses, or glucocorticoid steroids, may affect HIV replication and gene expression. Presumably, they also could affect HERV expression. The HIV-1 LTR (Fig. 5) consists of three functionally discrete regions (U3, R, and U5). Transcription initiation occurs at the U3-R boundary in the 5′ LTR. Table 4 lists the host cell factors

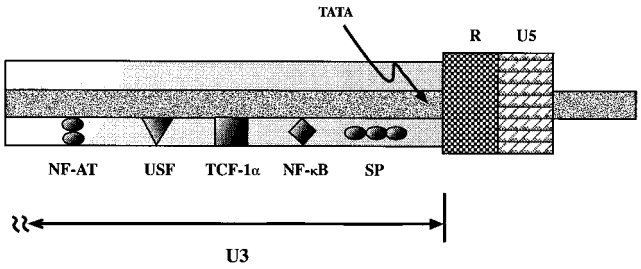

FIG. 5. Expanded diagram of the 5′ LTR of HIV-1 schematically representing the binding of regulatory cell factors to the U3 region. The major "modulatory" and "core" host cell factors are defined in Table 4. HIV and HTLV, possibly because of their complex regulatory genes, appear to be critically sensitive to host cell factors compared with the simpler retroviruses. Therefore, such host cell factors may be of major importance in the cell and tissue specificities of the expression of some families of HERVs.

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TABLE 4. Host cell factors that bind to the HIV LTR

Element category	Definition	Function
Modulatory		
AP1	Activator protein 1	Cellular transcription factor that regulates the response of cellular genes to growth factors, phorboesters, etc.; may positively or negatively affect HIV gene expression
COUP	Chicken ovalbumin upstream promoter	Belongs to the steroid-thyroid hormone receptor family; may positively or negatively affect HIV gene expression
NF-AT	Nuclear factor of activated T cells	Binds to IL-2 gene promoter and may stimulate HIV gene expression
USF	Upstream stimulating factor	May increase or decrease HIV gene expression; effect depends on other participating cellular factors
TCF-1α	T-cell factor 1α	T-cell-specific cellular factor; effects on HIV gene expression unclear
NF-κβ	Nuclear factor-κβ	Broadly distributed in cell types; regulates many genes
Core		
SP-1	Cellular transcription factor SP-1	Stabilizes binding of cellular factors to HIV LTR
Initiator	Initiator-element-binding proteins	Transcriptional regulators; precise role not clear
TATA	The TATA box	Binds TATA binding factor to yield an initiation complex with RNA polymerase II that includes transcription factors II A–H and plays a major role in upregulating HIV gene expression

that bind to HIV-1 LTRs and their role in HIV-1 gene expression, if known.

HTLV-1 Replication

The HTLV family consists of HTLV-1, HTLV-2, simian T-cell leukemia virus type 1, and bovine leukemia virus. The biological profile of the HTLV viruses is distinctive as noted below. HTLV-1 will be regarded as the prototype and will be discussed with emphasis on regulatory mechanisms in virus replication and gene expression. The HTLV genome is complex, like that of HIV-1 (Fig. 4) but somewhat shorter (9.0 kb). It encodes two transregulatory proteins, Tax and Rex, that are the functional counterparts of HIV-1 Tat and Rev, respectively. The pattern of HTLV-1 replication, with discrete early and late phases, is basically analogous to that described for HIV-1.

The early RNA transcripts of integrated provirus DNA are characterized by a predominance of spliced RNAs that code for Tax and Rex. As the amount of Rex increases, less spliced RNA is transported to the cytoplasm for translation, and the synthesis of genomic RNA and structural viral components predominates. Tax functions transcriptionally, while Rex functions posttranscriptionally. The mechanisms of HTLV-1 replication, and regulation of gene expression, recently were reviewed by Cullen (37), Green and Chen (61), and Sugamura and Hinuma (176). The biological profile drawn up for the HTLV viruses by the last authors is particularly informative and corrects the considerable confusion in the HTLV literature concerning the origins, distribution, and pathology of HTLV-1 infection.

Tax is of particular interest because its activity in *trans* is not restricted to the regulation of HTLV-1 genes, nor is it necessarily cell type or species specific. Tax appears to possess the capacity to affect cellular gene expression in a normal or pathologic context; hence, its biological activity seems to be considerably broader than that of Tat. The target sequence of the Tax protein (208) is a set of three 21-bp repeats in the HTLV-1 LTR (referred to as *trans* response elements [TRE] in some publications). However, the Tax protein does not bind directly to these sequences. Instead, members of the CREB/ATF family (208) of cellular DNA-binding proteins bind to the 21-bp repeats because such sequences contain the CREB/ATF binding motif. The Tax protein then binds to the CREB/ATF proteins to yield the final functional transactivating entity.

The finding that the *tax* gene product complexes with members of the CREB/ATF cell-binding proteins suggests cell-gene activation capabilities. Such activations have been found. For example, Tax activates cellular transcription factor NF-κβ (4), which in turn activates IL-2 (5) and IL-2 receptor genes (104, 166), thus establishing a possible base for autocrine-paracrine stimulation of cell growth. Whether such a mechanism is involved in the malignant transformation of cells is unknown. However, there are other ramifications of such transactivational activities. Thus, Tendler et al. (185) reported that HTLV-1 transactivation of IL-2 induced immune activation of HTLV-1-associated myelopathy. Such *trans* activity is not limited to HTLV. Ensoli et al. (47) reported that the Tat protein of HIV-1 stimulated the growth of cells derived from Kaposi's sarcoma lesions.

Rex is a 27-kDa phosphoprotein most abundant in cell nucleoli. Rex binds to a unique product of the R region of the 3′ LTR and, like Rev, acts as a molecular chaperone, causing a switch from the early to late phase of viral replication; i.e., more singly spliced and unspliced RNAs are transported to the cytoplasm for translation into viral structural entities or to serve as genomic RNA. Rex binds to a virus-coded RNA called the Rex response element, an event essential for its activity. Interestingly, Rex can replace Rev in HIV-1 replication but not vice versa. Cullen (37) makes the interesting point that Rex can rescue a Rev-deficient HIV-1 provirus.

HERVs

We define endogenous retroviruses in a classical context. They are integrated into the host genome and transmitted by Mendelian mechanisms; may be complete provirus transcripts that ordinarily are not expressed as infectious viral particles; may be defective in a given gene function(s) and unable to complete a full cycle of replication; may be expressed spontaneously, but infrequently, as complete infectious virions; may be inducible by exogenous agents such as X-irradiation, inhibitors of protein synthesis, halogenated pyrimidines, inflammatory cytokines, or steroids; may be rescued by complementation or by cocultivation with indicator or NP cells; ordinarily are not horizontally transmissible; and may act as insertional mutagens. Full-length HERVs may share any number of these characteristics.

HERVs can be detected in a number of ways, each having its

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TABLE 5. Detection by thin-section electron microscopy of retrovirus particles in human tissues

Tissue or cell type	Disease category of tissue donor	Morphologic type	Reference(s)
Fibroblast or fetal	Normal	Type C	125, 145, 154
Embryonic cell line	Embryonic carcinoma	RTV[a]	20, 21, 154
Platelets	Primary proliferative polycythemia	RTV	18
Peripheral blood monocyte	Sjögren's syndrome	Type A	57, 62
Cultured blood monocytes	Multiple sclerosis	RTV	152
Placenta	Normal	Type C	43, 83, 86, 192
Placenta	SLE	Type C	43, 77, 144
Oocytes	Normal	RTV	101, 135
Teratocarcinoma cell lines	Teratocarcinoma	Type C	21, 97, 108, 109
Breast cancer cell lines	Breast cancer	Type B	3, 48, 140
Psoriatic lesions, blood lymphocytes, epidermal cells	Psoriasis	RTV	78, 79

[a] RTV, Retrovirus-like particles.

limitations. For the most part, they include the following: detection by thin-section electron microscopy; detection of retrovirus antigens associated with purified viral preparations; measurement and characterization of RT activity; detection of HERV gene sequences in cells or tissues; detection of HERV RNA transcripts or their translation products; cocultivation techniques with indicator cells; transfections assays; and attempts at phenotype mixing designed to broaden HERV indicator cell host range.

Electron Microscopy Studies

A search of the literature was made to gauge the frequency (Table 5) of the detection, by thin-section electron microscopy, of retroviruslike particles in human tissues or established human cell lines. The finding that type C viruses (125, 145) were associated with normal fetal fibroblasts cultured in vitro, or embryonic tissues, is consistent with observations reported in the decade 1960 to 1970. Whether such viruses were endogenous to fetal tissues or were of placental origin is unknown. Whatever the case, they are real. Retroviruslike particles were detected (18) in patients with primary proliferative polycythemia and in a blood dyscrasia (62) characterized by $CD4^+$ T-cell deficiency. The latter appeared to be IAPs. Similar particles were found associated with salivary tissue extracts (57) in patients with Sjögren's syndrome. Retroviruslike particles also were detected (152) in patients with multiple sclerosis.

Kalter and his coworkers were among the first to report (86, 87) the occurrence of type C virus particles in the placentas of baboons, chimpanzees, and humans. Such virus particles are of interest for several reasons. They are different from classic type C viruses because they lack a space between their nucleocapsids and envelopes. Because they are not transmissible in vitro from human cell to cell, Dirksen and Levy (43) make the cogent suggestion that they may be the human xenogenic counterpart of the mouse xenotropic viruses. The significant finding that a specific and potent inhibitor of RT activity copurified with such virus particle fraction was made (133, 192). Type C viruses are not randomly distributed in the placenta (43) but are most abundant at the basal surface placental syncytiotrophoblast. Various reports in the literature describe the association of type C viruses with SLE. In the Dirkson and Levy study (43), placentas from 9 healthy women and from 10 women with SLE were thoroughly analyzed by thin-section electron microscopy for the occurrence and frequency of type C virus particles in various placental tissues. Virus particles were found in the placentas of four healthy women and in those of three of the women with SLE. These results were not substantially different from the reports by Kalter et al. (86) or Vernon et al. (192), who analyzed healthy subjects, nor did they differ materially from those of Imamura et al. (77), who also studied placental tissues obtained from women with SLE. Considered together, these findings neither include nor exclude placental type C viruses as etiologic participants in SLE.

Studies to determine whether type C viral particles occurred in male reproductive tissues were carried out. Bronson et al. (20, 21) and Kurth et al. (97) found that testicular teratocarcinoma cell lines infrequently produced type C virus particles unless exposed first to an inducing agent such as 5-iodo-2′-deoxyuridine, dexamethasone, or dimethyl sulfoxide. Cell lines differed in responsiveness. The Tera-1 and GH lines were readily inducible, while Tera-2 was not. In a very informative study, Löwer et al. (108, 109) used long-term explant cultures of human teratocarcinoma-derived cell lines to analyze virus production. Few virus particles were found unless cultures were induced with 5′-iodo-2-deoxyuridine, dexamethasone, or dimethyl sulfoxide. Such viruses could not be transmitted by cocultivation with a diverse spectrum (dog, cat, mink, bat, tupaia, marmoset, African green monkey, human amnion, and human rhabdosarcoma) of indicator cells. Such teratocarcinoma viruses were not related antigenically to simian, cat, mouse, or bovine retroviruses. The authors suggested that the teratocarcinoma viruses made up a unique group of HERVs.

On the basis of a large body of information showing that MMTV caused breast cancer in mice, there is ample reason to presume that at least some forms of human breast adenocarcinoma may have a retrovirus etiology. Early work was fraught with the difficulty of identifying retroviruslike particles resembling the type B particles of MMTV in human breast adenocarcinomas or human milk. The current status of the field can be garnered from three representative publications. Thus, Al-Sumidaie et al. (3) thoroughly documented the occurrence in situ of type B particles in monocytes, macrophages, and giant cells of breast cancer patients. Faff and his associates (48), in an elegant study, provided unequivocal evidence that the T47D cell line produced type B particles, that the genome for virus production was endogenous, and that virus production was stimulated by steroids. Similarly, Ono et al. (140), also using the T47D cell line, reported that the expression of an 8.8-kb full-length genome of HERV-K endogenous to T47D cells was enhanced by progesterone and estradiol. Considered together, the described results establish that type B retroviruses superficially similar to MMTV are endogenous to the human genome.

SPECIFIC HERVs

For the most part, the HERVs isolated to date have been derived from either reproductive tissues, somatic cells, circulatory lymphocytes, or monocytes. Limited work has been done to determine the degree to which the HERVs derived from these categories of tissues or cells are related. We comment on possible interrelationships below as appropriate. In the sections that follow, HERVs were grouped according to whether they most likely fit into the classic type C or B category and according to copy number. We found it difficult to deal with the category of DNA sequences that are intermediate between

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retrotransposons and retroposons. HERV S71 is a classic example. Thus, the protease and RT domains of *pol* are missing; *gag* is truncated; *env* is absent; and there is a 3′ LTR but no 5′ LTR (198). That is, S71 is not a provirus. Therefore, S71 and elements like it will not be discussed. In addition, information for some HERVs is still too limited to warrant consideration. Because of their importance, HERVs possibly related to HTLV-1 and HIV-1 will be considered separately.

HERV 4.1 (HERV-E): Multicopy Type C

Martin and coworkers (118) used as a probe a 2.75-kb segment of African green monkey DNA that hybridized to murine leukemia virus (MuLV; AKR mouse ecotropic leukemia virus) and endogenous baboon virus (BaEV). The described probe detected related sequences in human brain fragments and in a human DNA library. Clone 4.1 made up a full-length provirus. The related clone 51.1 approximated a retroposon. The complete nucleotide sequence (161) of HERV 4.1 was determined. The 3′ LTR is 449 bp and the 5′ LTR is 495 bp, with 95% homology between them. Genomic DNA consisted of 8,806 bp organized into *gag*, *pol*, and *env* sequences interspersed between the two LTRs. On the basis of deduced amino acid sequences, HERV 4.1 *gag-pol* sequences exhibited approximately 40% homology to Moloney MuLV. Termination codons and point deletions in the genome indicated that HERV 4.1 was replication defective. However, long ORFs in the *pol* and *env* regions suggest that they might be expressed. The fact that the 18-bp primer binding site for tRNA does not match $tRNA^{Pro}$ (found in most mammalian type C retroviruses), but is homologous to tRNA glutamine, is unusual and has suggested a primordial origin of HERV 4.1.

Rabson et al. (158) carried out experiments to test whether mRNA transcripts of the HERV 4.1 genome could be detected. LTR and *env* segment probes were used to test for RNA transcripts in human liver, placenta, spleen, a colon carcinoma line, and a virus-free HTLV T-cell line. Human placenta contained substantial amounts of a 3.0-kb RNA that hybridized to both probes. A 1.7-kb polyadenylated RNA that hybridized to the *env* probe also was found in placenta. The liver, spleen, and human colon cancer cell lines contained lesser amounts. Variable amounts of 3.6- and 2.2-kb RNAs were found, particularly in the colon cell line. When *gag* or *pol* probes were used, no RNAs were detected. A 6.8-kb RNA species that hybridized to *gag-pol* and *env* probes was found in the HTLV cell line. Rabson et al. (158) also found that the described line of T cells contained a 4.8-kb RNA species that hybridized to *gag*, *pol*, and *env* probes. Thus, most tissues and cell lines tested expressed RNAs transcribed from a defective full-length provirus genome. One wonders whether this was accomplished through a mechanism of complementation.

RTVL-H (HERV-H): Multicopy Type C

While analyzing the human β-globin gene, Mager and Henthorn (116) discovered repetitive DNA sequences that exhibited similarities to endogenous retroviruses but that had a $tRNA^{His}$ binding sequence. The retroviruslike sequence therefore was designated RTVL-H. Computer searches by the authors "failed to disclose significant homologies to known retroviruses including HTLV I or II, mouse IAPs or HERV 4.1." Copy numbers were estimated to be 860 per haploid genome, thus indicating the existence of a large family of such retroviruslike sequences.

Mager and Freeman (115) cloned several prototypic members of the RTVL-H family and characterized one (RTVL-H2) in some detail. The provirus structure of RTVL-H2 consisted of 5,813 bp, including both LTRs. The 5′ LTR was 450 bp; the 3′ LTR was 451 bp. There was only a 4% mismatch between them. The LTRs had correctly positioned promoter and polyadenylation signals, with a primer binding site just inside the 5′ domain that was homologous to $tRNA^{His}$. Genomic RNA lacked long ORFs. Thus, RTVL-H2 appeared to be a severely truncated provirus. The authors reported that the *pol* region of RTVL-H2 had "segments similar to Moloney MuLV polyprotein and that RTVL-H2 *gag* had a segment with significant homology to a region of the *gag* protein p30 type C BaEV." The authors also reported putative homologies between RTVL-H2 and HTLV-1 and -2. In a subsequent publication, Wilkinson et al. (200) reported the isolation of "RTVL-Hp" that contained a functional domain in the *pol* region that had not been detected in previous isolates. The authors make the reasonable comment that among the many copies of RTVL-H in the human genome there appears to exist a "small functional subfamily of RTVL-H elements in the human genome."

The diversity of the RTVL-H family is further illustrated by the report of Hirose et al. (69). cDNA libraries were prepared from poly(A′) RNA obtained from various tissues and cell lines by using oligo(dT)- or $tRNA^{His}$-derived oligonucleotide primers. PCR was used for amplification with appropriate primers. Several RTVL-H clones containing *env*-related sequences were isolated. One clone (RGH 2) approximated a provirus in size (8.7 kb in length), with both 3′ and 5′ LTRs. The *env* gene had an ORF capable of encoding 570 amino acid residues. Comparison of the deduced amino acid sequence of the *env* region of clone SA49 with the immunosuppressive peptide regions (p15E) of various conventional retroviruses revealed homologies of 75 and 71.2% for Mason-Pfizer monkey virus (type D) and reticuloendotheliosis-associated virus (type C), respectively, and ca. 58% homology to both feline leukemia virus and Moloney MuLV. Thus, HERV SA49 is a mosaic of sequences related to both type C and D retroviruses.

Wilkinson et al. (199) used several RTVL-H probes to detect RNA expressed in a spectrum of human cell lines that had been established from various malignancies. Transcription patterns differed significantly. However, a unit-length 5.6-kb transcript was found in HEp-2, two bladder cancers, and one embryonic kidney cancer cell line. A 3.7-kb transcript also was found in various cell lines. Interestingly, no transcripts were found in cell lines derived from patients with myelogenous leukemia or breast or myeloma cancer or in an Epstein-Barr virus lymphoblastoid cell line. When primary tissues were tested, six amnion and four of six chorion samples expressed 5.6-kb RNA. A 3.7-kb band also was found in three amnion samples. One 1.5-kb RNA transcript was found in two chorion samples.

In similar studies, Hirose et al. (69) detected RNA transcripts of several categories. RTVL-H RNAs were highly expressed in tissues from a lung squamous cell carcinoma, two lung cancer cell lines, and two teratocarcinoma cell lines. The important finding was that expression was enhanced in some cell lines by treating cells with the hypomethylating agent 5-azacytidine. Overall, three classes of transcripts to the RTVL-H *env* gene were found. One class hybridized exclusively to the $tRNA^{His}$ probe or *gag* probes. The second were transcripts that hybridized only to the 3′-terminal region of *env*. The third class hybridized to major portions of *env* genes. Most interestingly, an 8.2-kb transcript that was essentially genomic in size also was found.

To summarize, it is apparent that there is a great diversity of RTVL-H retroviruslike sequences in the human genome. Many of them are highly truncated. Many are mosaics. Many are detectable (60) in apes and Old World monkeys (OWM)

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and in some New World monkeys (NWM; marmosets) but not in rodents. Their unique $tRNA^{His}$ primary binding site suggests a primordial origin. On the whole, not enough work has been done to permit grouping them into well-defined categories. Their high copy number, along with detection of a spectrum of RNA transcripts, indicates that complementation and full expression may occur under circumstances yet to be described.

ERV-1 and ERV-3 (HERV-R): Single-Copy Type C

A fragment of a chimpanzee *pol* probe (CH2) was used by Bonner et al. (16) to screen a human genomic library for homologous sequences. A *pol* sequence of human DNA was found highly homologous (99%) to the CH2 *pol* probe and was used for screening. DNA sequence homology to the *gag* (51%) and *pol* (57%) genes of Moloney MuLV also was found. Subsequently, O'Brien et al. (137) were able to assign this DNA sequence (named ERV-1) to human chromosome 18. Since ERV-1 lacks an authentic *env* gene sequence and a 5′ LTR, occurs as a single copy, and very likely cannot be expressed, it will not be discussed further.

The HERV ERV-3 (HERV-R), with a primer binding site homologous to that of $tRNA^{Arg}$, was discovered by O'Connell et al. (138) when a human fetal liver DNA library was screened with a 0.75-kb restriction fragment from the 3′ end of chimpanzee clone CH2 and a fragment of the LTR of baboon virus isolate M7 (BaEV). ERV-3 is an authentic, full-length HERV, 9.9 kb long, that has been mapped to chromosome 7. The ERV-3 genome conforms to a typical retrovirus structure. Each LTR had ca. 590 bp. ERV-3 *gag* and *pol* sequences exhibited homologies to other mammalian type C retroviruses, but the significance of the homologies is not clear.

Kato et al. (89) analyzed the expression of ERV-3 in placental tissues. Three polyadenylated RNAs, 9, 7.3, and 3.5 kb long, were abundant in the human placenta chorion. All were spliced mRNAs. The 3- to 5-kb RNA was a typical subgenomic proviral mRNA. However, none of the transcripts included a full-length genomic RNA. Cohen et al. (32) analyzed ERV-3 RNA expression in normal and malignant tissue and cells. These investigators were particularly interested in determining whether the LTR glucocorticoid regulatory sequences upstream from the promoter might be implicated in ERV-3 RNA expression. The analysis was facilitated by the fact that ERV-3 occurred only as a single copy in human DNA. Cohen et al. (32) found three major mRNAs (9.0, 7.3, and 3.5 kb) expressed in first-trimester placental chorionic villi. By contrast, embryonic tissue expressed only the 9.0-kb RNA transcript and low amounts of the 3.5-kb species. All three mRNAs were spliced. The 3.5-kb mRNA was a typical subgenomic spliced proviral message expressing *env*, but not *gag* or *pol*, sequences. There was an absence of ERV-3 expression in choriocarcinoma tumor cells, thus invoking a suppressive mechanism. Levels of expression in various tissues were disparate. The highest level of expression was in placental chorionic villi. Other tissues (thymus, breast, gallbladder, glioma, pancreas, lung, etc.) expressed mRNA at levels of 10 to 30% of the placental villi. The 7.3-kb mRNA was confined to placental tissues. Taken together, these results suggest tissue specificity of expression probably mediated by steroid hormones. The definitive experiments of Boyd and his associates (17) clarified the question of steroid responsiveness. These investigators found that ERV-3 *env* expression did not occur in undifferentiated cytotrophoblasts. ERV-3 RNA expression occurred when cytotrophoblasts differentiated into syncytiotrophoblasts, a process responsive to β-human chorionic gonadotropin. These authors made the cogent suggestion that β-human chorionic gonadotropin regulated ERV-3 RNA expression through the regulation of the cell factors required for such expression.

MMTV-Related HERVs

MMTV-related HERVs are considered together because of their possible relevance to human mammary carcinoma. The central issue is whether such HERVs studied to date, which have been reported as MMTV-like, in fact possessed the physical and molecular characteristics of MMTVs. These characteristics include the following: type B particles with typical physical organization; whether particulate fractions purified from tissues or cells have a buoyant density, RT activity, and antigenic moieties typical of MMTVs; genome composition and organization characteristic of MMTVs; LTR receptors for steroid hormones; tissue specificity of expression; and responsiveness to hormone stimulation comparable to that of MMTV.

Convincing evidence was published by Keydar et al. (90), who showed that human breast cancer line T47D produced retroviruslike particles in a steroid-dependent (17-estradiol and progesterone) fashion. Preliminary evidence indicating that the retroviruslike particles produced possibly shared significant antigenic determinants with MMTV was obtained. However, these investigators (169) were unable to document a significant antigenic relationship between the retroviruslike particles produced by T47D and those produced by MMTV gp52 antigen (the major glycoprotein envelope antigen of MMTV). Subsequently, Ono et al. (140) studied the expression of HERV-K full-length polyadenylated RNA (8.8 kb) in a spectrum of established human cell lines, including T47D. Expression was stimulated in T47D cells when the correct regimen of estradiol and progesterone was used. Dexamethasone had no effect. Expression was stimulated by estradiol or progesterone in HeLa, HEp-2, or a line of melanoma cells.

Several investigators have carried out studies to determine sequence homologies between human and MMTV DNA. Often searches were done with MMTV *pol*-specific probes. Thus, Deen and Sweet (39) employed MMTV *pol* gene probes to screen human DNA libraries for homologous sequences. They found that the human haploid genome contains ca. 30 to 40 such sequences. For one of these (HM16), the *pol* gene was sequenced, and the sequence was compared with that of MMTV *pol*. The nucleotide homology was ca. 52%.

May and her associates have carried out extensive studies designed to evaluate the occurrence of MMTV-like retrovirus sequences in human tissues and cells. One publication is discussed as representative. Thus, Franklin et al. (52) screened a human DNA library constructed from breast cancer line MCF-7, using an MMTV *gag-pol* probe. Sequences that were cloned from this library of MMTV-related DNAs made up nine distinct groups. The *gag-pol* regions of a single clone selected from each of the nine groups were hybridized to RNA prepared from five human breast cancer cell lines, two nonbreast cancer lines, and placental tissues. Probes from six of nine groups detected large RNAs. Some might represent full-length proviral RNAs. Transcripts ranging in size from 1.2 to 12 kb were detected by probes from seven of nine groups. Two probes detected RNA transcripts in one breast cancer line only. These results once again show how the probes used to detect HERV sequences yield diverse results. Restriction maps were determined for representative recombinants of each of the nine groups (NMWV-1 through NMWV-9). Group 1 of these MMTV-related sequences comprised 64% of the isolated clones. Recombinant C5 contained the HLM-2 sequence described by Callahan et al. (23). Clones Ab and E11 con-

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tained sequences homologous to those of the HM reported by Deen and Sweet (39). RNA transcript expression was greatest in placental tissues. These authors also stated that RNA expression of NHWV-4 was progesterone sensitive. Considered together, these findings again demonstrate a great diversity among human DNA sequences that hybridized to the MMTV *gag-pol* probes. The diversity of the sequences detected greatly complicates experimental analysis and interpretation of results. An approach to solving such difficulties is illustrated by the studies of Medstrand and Blomberg with the HML family of HERVs discussed next.

The HMLs studied by Medstrand and Blomberg (120) originally were detected in peripheral blood monocytes (121) of healthy individuals. The three subclasses detected showed sequence homologies to Martin et al.'s HERV-4.1 (HERV-E), Mager et al.'s RTVL-H (HERV-H), and teratocarcinoma-derived HERV-K10. There are two interesting features of these findings: that HERV-K DNA sequence homologies were found in peripheral blood monocytes of healthy individuals, and the occurrence of a diversity of sequences in a fully differentiated blood cell type.

Medstrand and Blomberg designed a cogent experimental approach to gain insights into their original findings (120). They extracted RNA from human peripheral blood monocytes and reverse transcribed it to DNA. PCR was used to amplify the DNAs thus obtained, using conserved sequences of reverse transcriptase as primers in PCR amplifications, viz. 297-bp segments of mouse or hamster IAP sequences, MMTV, and Mason-Pfizer monkey virus. The products obtained were cloned and used as probes. Hybridization studies revealed sequences that were divisible into six HML subgroups that differed by as much as 25%. Five of the subgroups were most homologous to MMTV. One showed the closest homology to IAPs. The pattern of expression of RNA transcripts in various tissues was diverse but suggested tissue specificity. Probes HML1-6 hybridized to eight normal tissues, with highest expression in the lung. Transcripts to HML1-HML3 and HML6 sequences occurred more frequently than did those to HML4 and -5. Transcripts ranged in size from 1.4 to 9.5 kb, with some possibly representing full-length proviral transcripts. HML2 contained DNA sequences common to HM16 and HERV-K10. The degrees of relatedness between HML1 to HML6 and HERV-K10+ was not determined.

To summarize, from the findings outlined above, it is clear that the so-called MMTV-related sequences described appear to represent a portion of a sequence mosaic shared by IAPs and HERV-K10 and occur in relatively high copy number. This result correlates with the use of highly conserved sequences of *pol* as probes. While the results of Keydar et al. (90) clearly show that retrovirus particles are associated with the T47D human breast cancer cell line, they do not provide evidence that they are related to MMTV. Contrary to reports in the literature, the immunologic studies of Segev et al. (169) suggest a trivial antigenic relationship to the particles detected by Keydar et al. (90) and MMTV gp52. The results obtained by Franklin et al. (52) and Medstrand and Blomberg (120) clearly establish that MMTV-like sequences in human DNA show broad diversity.

HERV-K10 Family: Multicopy Type C

Human teratocarcinoma-derived HERVs (HTDV-H) were observed (15) as type C virus particles in cell lines established from human teratocarcinomas. Because such HERVs bind $tRNA^{Lys}$ to their primer binding sites, they have been designated HERV-K. They make up a large multicopy family, have been found in numerous tissues, and are authentic prototype endogenous proviruses. They are considered separately because of their abundance in and association with human reproductive tissues. Possibly they are associated with other tissues or cells in a less conspicuous form. Boller et al. (15) established that HERV-K codes for at least one HTDV.

Several teratocarcinoma cell lines have been well characterized. Thus, the GH line (109) is an avid producer of virus particles and is inducible by exogenous agents, as is Tera-1. The Tera-2 cell line is a poor virus particle producer and is not readily inducible. Löwer et al. (108) found full-length HERV RNA transcripts in GH cells and spliced RNAs analogous to those of classical endogenous proviruses. Such transcripts had the potential to code for a complete set of viral structural proteins. These investigators also found that slightly different *gag* gene sequences occurred among seven tested clones. This suggested, in concordance with the detection of ca. 50 copies of the HERV-K provirus per haploid human genome, that multiple HERV genomes were transcriptionally active, thus indicating functional gene complementation.

The results of studies of HERV-K expression in human buffy coat cell preparations are of interest for several reasons. Brodsky and his coworkers (19) analyzed HERV-K *pol* gene expression in buffy coat leukocyte preparations obtained from three groups of patients (three with polycythemia vera, three with chronic myelogenous leukemia, and two with acute myelogenous leukemia) and one group of seven healthy adults. Leukocytes from the latter expressed a variety of HERV-K *pol* sequences. Patients with myelogenous leukemia expressed a common defective *pol* sequence. Four different *pol* sequences were expressed in the leukocytes from patients with polycythemia vera. These results suggest the following: HERVs of possible reproductive tissue origin may occur unexpectedly in nonreproductive tissues or cells, and the diversity of *pol* sequences in HERV-K proviruses may permit an override of their termination codons by gene complementation.

In a classic paper, Ono et al. (141) determined the complete nucleotide sequence of HERV-K10+. These investigators compared the nucleotide sequence of HERV-K10+ with those of Syrian hamster IAP, simian immunodeficiency virus (type D), squirrel monkey retrovirus (type D), MMTV (type B), and RSV (type C). Standard (141) HERV-K10 had a genome of 9,179 bp, with a 290-bp deletion between *pol* and *env*. The prototype HERV-K10+ lacked this deletion; hence, its composite genome was 9,469 bp. Thus, K10+ is the authentic HERV-K10; i.e., it is representative of a class of endogenous proviruses. Both LTRs are 970 bp. The primer binding site binds $tRNA^{Lys}$, which also is the case for visna virus and simian retrovirus-1. Immediately upstream from the LTR enhancer is a segment corresponding to a glucocorticoid responsive element analogous to that found in the MMTV LTR. The reading frame of the coding strand contains six large ORFs (ORF 1 through ORF 6) not overly interrupted with termination codons or frameshift mutations, i.e., capable of being expressed. ORFs 1 and 2 code the *gag* gene. ORF 4 codes the *pol* gene. ORFs 5 and 6 code the *env* gene. Knowledge of the nucleotides that make up these gene sequences made it possible to determine their deduced amino acid sequences. In turn, it was possible to make accurate comparisons with other test retroviruses. Thus, the integrase domain of the *pol* gene of HERV-K10+ exhibited higher homology to MMTV than to simian retrovirus-1 or IAP-18. The *pol* RT domain was equivalent in homology to type A, B, and D retroviruses. Comparative analyses of the *env* nucleotide sequences disclosed a greater degree of homology between HERV-K10+ and MMTV than between HERV-K10+ and simian retrovirus-1

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and IAP-18. Thus, Larsson's categorization (100) of HERV-K10+ as a multicopy mosaic-type endogenous provirus is accurate and portrays correctly its molecular constitution. Therefore, it is inappropriate to consider it an MMTV-like, IAP-like, or type D-like HERV; i.e., it is a distinct entity. Notwithstanding these considerations, HERV-K10+ is a classic defective endogenous retrovirus that exhibits considerable genetic variability, occurs intact or otherwise in various tissues or cells, has glucocorticoid receptors, and because of its genetic integrity, should be expressed in tissues or cells under appropriate conditions. Accordingly, the report by Mueller-Lantzsch et al. (128) is of particular interest because these studies established that HERV-K10 encoded a full-length Gag protein (73 kDa) and a functional protease in *Escherichia coli*.

HERV-K(C4) Family: Multicopy Type C

Members of the HERV-K(C4) family were found by Tassabehji et al. (182) during restriction mapping studies of human DNA. The HERV-K(C4) family is made up of 10 to 50 members, is dispersed over several chromosomes, and shares significant sequence homology with HERV-K10+. One member of the family, HERV-K(C4), was studied in detail (182). HERV-K(C4) is located in intron 9 of the complement C4A6 gene but also is found in some C4B genes. Such sequences appear to be principal contributors to the interlocus and interallelic heterogeneity of C4 genes. HERV-K(C4) sequences occur in the DNA of gorillas, chimpanzees, and orangutans and in at least one species of macaque (*Macaca fascicularis*). Homologous sequences were not found (182) in marmosets (*Leontopithecus saguinus* and *Cebuella pygmaea*), thus suggesting that HERV-K(C4) sequences were inserted into the primate genome after the divergence of NWM and OWM. Members of the HERV-K(C4) family clearly are distinct from those of the HERV-K10 family. Thus, HERV-K(C4) is truncated, having a complete nucleotide sequence of only 6,357 bp. The 5′ and 3′ LTRs also are truncated, consisting of 546 and 548 bp, respectively, but with a divergence of only 7.8%. The *pol* and *env* sequences of HERV-K(C4) exhibit 64.5 and 60.3% homology, respectively, with the corresponding HERV-K10+ sequences. HERV-K(C4) also appears to have significant sequence homology to *pol* sequences of HERVs HML3, HML5, and HML6 (182). Multiple termination codons occur in the various ORFs of HERV-K(C4). No data on HERV-K(C4) gene expression have been reported. The occurrence of members of the HERV-K(C4) family in the C4A and C4B genes of the class III region of the human HLA complex invites speculation on possible biological and immunological implications. For example, it is conceivable that insertions of single or multiple copies of HERV-K(C4), or other members of this family, into C4A or C4B sequences could alter important immunologic reactivities, such as complement-dependent cell-mediated cytotoxicity, or engender autoimmune reactivities by alteration of HLA autoantigens. Moreover, regulatory sequences in the LTRs of HERV-K(C4) entities might affect the expression of C4A, C4B, or HLA genes.

ERV-9 Family

La Mantia et al. (99) derived from NT2/D1 teratocarcinoma cells a repetitive sequence (pHE.1) "defining a new family of endogenous retroviral sequences" that they named ERV-9. The NT2/D1 cells, reported to have been cloned and therefore not composed of a spectrum of genetic or phenotypic variants, evidently were derived from the Tera-1 or Tera-2 line established originally by Löwer et al. (109) from a human embryonic testicular teratocarcinoma. As documented above (108), various lines derived from the original embryonic teratocarcinoma were known to differ significantly in their production of type C virus particles and also were known to contain multiple copies of HERV-K. ERV-9 HERVs appear to be severely truncated, although analysis of the 4.0-kb repetitive sequence disclosed the presence of ORFS "potentially coding for putative retrovirus-related *gag*, *pol*, and *env* proteins" (99). LTR-like sequences were composed of subrepetitive elements. ERV-9 RNA transcripts were 8, 2, and 1.5 kb long in undifferentiated NT2/D1 cells. When NT2/D1 cells were treated with retinoic acid, a developmental morphogen, NT2/D1 cells differentiated and ERV-9 RNA transcription appeared to be negatively regulated. The results of Loh et al. (107) indicated that inhibition of retrovirus expression may have been caused by a *trans*-acting regulatory factor present in normal or malignant embryonic cells. Contrarywise, Deragon et al. (41) reported that RT activity, and perhaps retrovirus particle production, was enhanced in NTera 2D1 cells by UV irradiation. In fact, this appears to be the only report in the literature documenting that UV radiation (200 ergs/mm^2) enhanced HERV expression.

RR HERV-I

Retinoic acid reactive HERV-I (RR HERV-I) was found (88) during studies of the retinoic acid inducibility of HERV transcription in subclones of a human ovarian teratocarcinoma. This HERV is of interest for several reasons: its mRNA is transcriptionally activated by retinoic acid; its primer binding site binds tRNA^{Ile}; and it shares significant *env* and LTR homologies with ERV-3 (tRNA^{Arg}). RR HERV-I occurs at 20 copies per haploid genome, is severely truncated (3,357 bp), and lacks *gag* sequences. At best, it is a borderline provirus. Nevertheless, it is of particular interest that retinoic acid treatment of parent clone 6 and its derivative, subline 9117, not only enhanced RR HERV-I RNA transcription but concomitantly increased susceptibility of these lines (ca. 10-fold) to infection by Gibbon ape leukemia virus. A possible linkage between these parallel events was not studied despite its inherent interest and evident significance. Retinoic acid enhancement of RR HERV-I expression was discussed primarily (88) in terms of enhanced promoter activity, a consideration of substantial importance. Whatever the mechanism(s), the described findings again emphasize the importance of cell factors in retrovirus and HERV expression. It is clear that retinoic acid can either enhance or suppress expression depending, among other things, on the cell lines used for studies and their genetic regulatory mechanisms.

HuERS and HuRRS-P (HERV-P)

Proline tRNA is the most common primer used by mammalian type C retroviruses. Two groups of investigators therefore used tRNA^{Pro} probes to screen human DNAs for HERVs. Harada et al. (63) used the term HuERS (human endogenous retrovirus sequences) to describe their HERVs, all of which had primer binding sites homologous to that of tRNA^{Pro}. The HERVs detected by Harada et al. approximated LTRs and made up three subgroups (P1, P2, and P3) but did not possess the structural or molecular features of endogenous proviruses.

In a superb piece of work, Kröger and Horak (95) used synthetic oligonucleotides complementary to tRNA^{Pro} to detect HuRRS-P (human retrovirus-related sequence-proline) in human DNA libraries. An 8.1-kb element (HERV-P) with 600-bp LTRs that had the structural and genetic characteristics of a classical provirus was isolated. There were 20 to 40 copies

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per haploid genome. Homology studies showed significant degrees of hybridization with DNAs of both OWM and NWM. The occurrence of homologies to these simian species led these authors to suggest that a "progenitor virus of these sequences entered the germ line of a distant human ancestor at least 45 million years ago" (95). In our words, it was primordial. It appeared to be unrelated to any of the HERVs discussed above.

HTLV AND HIV SEQUENCES IN HUMAN DNA: GENERAL CONSIDERATIONS

The occurrence of HTLV or HIV sequences in human DNA should reflect the evolutionary distribution (55) of these retroviruses and their subprimate or primate hosts; i.e., their occurrence should not be random. The reasons to believe this are as follows. In brief, prosimians, along with their primordial retroviruses or retrovirus sequences, spread from Africa to Asia and South America some 40 to 60 million years ago (44). After continental separation, such prosimians followed independent evolutionary pathways to become either OWM or NWM. New retrovirus sequences undoubtedly were acquired after separation and radiation of OWM and NWM progenitors. OWM and apes diverged ca. 30 to 40 million years ago and now represent 77 species of African monkeys and 13 species of apes (55). Chimpanzees and gorillas are found only in Africa. Their Asian counterparts, orangutans and gibbon apes, occur only in Southeast Asia. After radiation, Asian macaques also followed distinct evolutionary pathways and currently make up 17 species. NWM make up 51 species. Evidence for a discrete distribution of exogenous or endogenous viruses (55) among these groups is rather striking. For example, a horizontally transmissible leukemogenic retrovirus is confined exclusively to the gibbon ape. The only retroviruses isolated from NWM are the endogenous type C virus of the owl monkey and the endogenous type D virus in the squirrel monkey. No exogenous type C or D retroviruses have been found in NWM. Exogenous simian type D retroviruses, composed of five serotypes, are confined largely to Asian macaques and are capable of causing simian AIDS. The Asian type D retrovirus has never been isolated from any African monkey species. Simian immunodeficiency virus, a simian lentivirus capable of causing simian AIDS, appears to occur exclusively (55) in African monkeys, particularly in the *Cercopithecus* and *Cercocebus* genera. Simian immunodeficiency virus consists of at least 30 distinct strains and thus makes up a complex family. For the most part, simian immunodeficiency viruses do not cause disease in their natural hosts and do not occur naturally in Asian macaques or apes. It has been proposed (124) that contemporary simian immunodeficiency viruses and HIV emerged within the last several centuries from earlier simian immunodeficiency virus progenitors.

Simian T-lymphotropic virus type 1 is the simian counterpart (53) of HTLV-1. Nucleotide sequence analysis reveals up to 95% homology. Serologic studies (176) have revealed a remarkable pattern of simian T-lymphotropic virus type 1 infection. Among some troops of Japanese macaques (*M. fuscata*), serologic evidence indicates infection rates as high as 95%. However, serologic surveys of African and Asian monkeys and apes disclose considerable differences in the seropositivity among various test groups. One can only conclude that infection by simian T-lymphotropic virus type 1 is common in OWM or apes whether they are in Asia or Africa. By contrast, NWM appear to be free of simian T-lymphotropic virus type 1 infection. Results of serologic surveys of HTLV-1 infections in humans (105) revealed "unusual geographic clustering." Such discrete distributions correlate with the serologic surveys made in monkeys and apes, as noted above. Some 43,445 human serum samples (105) were surveyed for antibody to HTLV-1. Antibodies were detected among subjects of African origin in the Caribbean basin and in sub-Sahara African populations. Native American populations in southern Florida and Central America also were positive. The high frequency of infection among Japanese, particularly in the prefecture of Kyushu, was as expected; i.e., a high frequency of HTLV-1-like HERVs in *M. fuscata* and in Japanese residents in Kyushu may reflect an ancient animal-to-human transmission. Significantly, Chinese populations were essentially antibody negative. The occurrence of seropositive individuals at significant frequency in Arctic regions (163) was unexpected. Equally unexpected is the identification of HTLV-2 variants in a Guaymi population (146). Considered together, the described results suggest the following: HTLV-1 infection of humans appears to be focal while simultaneously being worldwide; HTLV-1 virus(es) appears to be transmitted horizontally without requiring an arthropod vector; probably humans and HTLV-1 have had a long evolutionary association; or possibly HTLV-1 variants, or HTLV-1-like retroviruses, that have not been detected to date occur in human populations.

HTLV-1- AND HIV-1-RELATED HERVs

Wong-Staal et al. (204) used four different probes to screen cell lines, established from patients with different lymphoid and nonlymphoid malignancies, for HTLV-related sequences. Two probes were 400 to 600 nucleotides long and were R-U5 or R-U3 specific; i.e., they would detect LTR homologies. A third large (6-kb) probe had LTR-*env-pol* sequence homologies. The smaller fourth probe (3 kb) had *env-pol* homologies. When established cell lines from Japanese patients with adult T-cell leukemia were screened, HTLV provirus sequences were found, averaging one to three copies per cell. Two patients provided even more interesting results. One was serum antibody positive to HTLV but had T cells that were not positive for HTLV. In another patient, a defective HTLV provirus that lacked an LTR was found. In these studies, an Israeli patient with peripheral diffuse lymphoma was HTLV genome positive, as was a Brazilian patient with mycoides fungoides. Overall, the data indicated that HTLV genomes were present in HTLV-infected patients from Japan, the United States, Brazil, and the Caribbean and that such genomes were highly conserved. However, a patient with hairy cell leukemia yielded a distinct HTLV designated HTLV-2. Control patients were, for the most part, negative. However, one of 10 control patients with myelogenous leukemia was HTLV provirus positive when fresh leukemia cells were tested (204). The results of Wong-Staal et al. (204) indicated the following: they showed a worldwide distribution of HTLV-1; infected cells contained, on average, one to three proviruses per cell; some proviruses may have been defective; some patients with antibody to HTLV-1 may have had malignant lymphoid cells not containing an HTLV genome; and retroviruses similar to but different from HTLV may have infected different patient groups.

The findings summarized above did not provide evidence of HTLV-1 germ line infection, the hallmark of endogenous viruses. The results of the studies by Perl et al. (151) provided such evidence. These investigators used a 1.5-kb probe that contained the 5′ LTR and entire *gag* gene of HTLV-1 to screen normal human lymphocyte DNA for HTLV-1 homologous sequences. They found that HTLV-related endogenous sequences (HRES) were detectable in such preparations. HRES

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1/1 sequences were present in all preparations but displayed significant polymorphism and made up three classes: a 5.5-kb fragment; a class that contained both 3.7- and 1.8-kb fragments; and a class containing all three fragments. The frequency of the three genotypes differed among various lymphocyte donors. HRES 1/1 appeared to occur primarily as a single copy number of ca. 5.5 kb containing two ORFs capable of encoding p25 and p15 proteins. The single LTR detected had the regulatory sequences characteristic of a typical retrovirus LTR. The complete HRES 1/1 sequence was "different from any known human or viral sequence" (151). The LTR sequence, however, displayed significant homologies to HTLV-1 and -2 and HIV-1 TAR. When studies of HRES 1/1 RNA transcription were carried out, a 6.0-kb transcript was found with one probe but not with another. Transcripts were found in established lines of human melanoma cells, a line of promyelomonocyte leukemia, Epstein-Barr virus-transformed cells, and normal placental cells. Considered together, these results suggest that HRES 1/1 is a truncated provirus missing an LTR with ORFs that can be expressed at the RNA level. However, copy number appears to be too low to permit complementation of essential gene functions.

As one might expect from the principles outlined above, no HERVs related to HIV-1 or -2 have been detected in the human genome to date. However, some DNA sequences related to the highly conserved domain of the HIV RT and gp41 have been found (73).

HERVs IN PLACENTA AND OOCYTES

The occurrence of type C particles in reproductive and embryonic tissues has directed attention (66, 83, 100, 143, 201) to their possible roles in developmental biology or as pathogenic entities. They are relatively abundant (87) in the placentas of Old World primates such as rhesus, patas, and cynomolgus monkeys and of baboons, chimpanzees, and humans. They also are present in the placentas of New World primates such as marmosets and squirrel monkeys. Those found in the placenta and in teratocarcinomas are, on the whole, responsive to steroid hormones. As HERVs, they often occur as full-length genomes comparable to conventional endogenous retroviruses and are expressed, particularly in the placenta, as full-length RNAs analogous to retrovirus genomic RNA or as spliced RNAs comparable to those that code for retroviral structural or regulatory elements. It is not clear how placental type C particles may be related to those found in human embryonic lung fibroblasts grown in vitro (145) or to those found rather abundantly in first-trimester human embryos (125) or human embryonal cells (154). However, it is clear (15) that at least one HERV-K codes for a teratocarcinoma-derived type C viral particle. As noted above, type C particles do not occur randomly in the human placenta but are associated primarily with the basal surface placental syncytiotrophoblast. Moreover, cell type specificity is evident; i.e., cytotrophoblasts are relatively free of type C viruses, but such particles become readily detectable as cytotrophoblasts differentiate into syncytiotrophoblasts. Considered together, these properties suggest rather stringent regulation of type C virus expression mediated by cell factors present at some phases of cell differentiation but not at others and whose occurrence is steroid sensitive.

Evidence for the occurrence of particles resembling type C viruses in human oocytes and follicular fluids is convincing. Larsson et al. (101) detected type C particles in oocytes of women given drugs to stimulate ovulation. Such particles were found frequently budding from the surface of oocytes. The occurrence of particle-bound RT activity characteristic of retrovirus particles correlated with the thin-section electron microscopy data. Confirmatory results were obtained essentially a decade later by Nilsson et al. (135). Budding type C viruslike particles were found on the surface membrane of unfertilized oocytes obtained from women given drugs to stimulate ovulation. RT activity and MuLV gp70-like antigens were found associated with the particle-rich fraction of follicular fluids. Considered together, these two studies show that type C virus particles, and possibly some corresponding antigens, can be found in unfertilized oocytes and in follicular fluids. Corresponding studies (202) carried out to detect type C virus particles associated with human sperm or seminal fluids have failed to yield clear-cut results.

EXPRESSION OF HERV ANTIGENS IN PLACENTAL TISSUES

Because HERVs often have stop codons and frameshift mutations in their ORFs, one often finds the statement that such ORFs cannot be expressed. At face value, such a statement is reasonable. However, it overlooks the possibility that such defects may be overcome, particularly when HERVs occur in high copy number, by at least three compensatory mechanisms: complementation, termination suppression, and translational frameshifting. These mechanisms are relatively well known and are discussed by Cann (25). For example, such ribosomal frameshifting is known to occur (71) for MMTV.

One of the most convincing lines of evidence indicating that HERVs are expressed in vivo lies in the detection of immunologic responses to putative HERV-encoded antigens. Thus, Hirsch et al. (70) used cell cytotoxicity and radioimmune assays to test various groups of patients for immune reactivity to BaEV and simian sarcoma virus (SSV) antigens. Cell-mediated cytotoxicity by peripheral blood lymphocytes to BaEV-infected cells was not detectable in 14 healthy nonpregnant subjects (9 were males). Specific cell-mediated cytotoxicity was found only in pregnant women with peak reactivity at the second trimester. Such reactivity diminished during the third trimester to disappear after delivery (test group of six women). Antibody responsiveness to BaEV antigens correlated temporally with cell cytotoxicity. However, most subjects exhibited a low level of immune reactivity to SSV-related antigens. The significance of these results is difficult to assess since Kurth et al. (98) had reported earlier that "sera of many normal humans possess precipitating activity for the small structural virus proteins (p28, p15, or p15E) as well as for the large envelope glycoprotein (gp70) of simian sarcoma associated virus and BaEV, and for the p30 of Moloney leukemia virus: avian oncornavirus proteins could not be precipitated."

In a subsequent study, Kurth et al. (97) used radioimmune assays to quantify the humoral immune response of teratocarcinoma patients to the *env* antigens (gp70) and the structural antigen (p30) of SSV, as well as transmembrane protein p15E of Friend leukemia virus. Control patients were those with malignancies other than teratocarcinomas or autoimmune or cerebral degenerative diseases. Patients with well-differentiated teratocarcinomas had the highest titer of antibodies to SSV gp70. Low antibody titers to p15E were found and were considered to reflect a response to p15E interspecies antigenic moieties. These results may represent an immune response to the HERVs known to be present in teratocarcinomas.

Several additional lines of evidence indicate that placental HERVs may be expressed as antigens. For example, Thiry et al. (187) used antisera to Mason-Pfizer monkey virus and BaEV to test for related antigens in the placenta (and other tissues and cells), employing a complement-dependent cyto-

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toxicity test. Cytotoxicity to trophoblasts prepared as suspensions from healthy placentas, to cord blood lymphocytes, and to cultured fetal tissues was demonstrable, provided that the last were propagated in vitro for a sufficient period of time and had been pretreated with 5-iododeoxyuridine. Employing a different approach, Suni et al. (178) used antibody to p30 of feline leukemia virus RD114 as a histochemical probe. They found p30-related antigen in 24 of 24 healthy placentas (6 to 42 weeks of gestation), in 10 of 10 choriocarcinomas, and in 10 of 10 "blighted ova," but none in fetal tissues. Antigen was confined largely to the cytoplasm of syncytiotrophoblasts. The antibody probe was blocked specifically by RD114 p30 protein. When 1,540 cord blood serum samples were tested for immunoglobulin G, 118 (7.7%) were positive for RD114 antibody. The occurrence of such antibodies in cord blood serum "had a highly significant correlation to complications during pregnancy and also correlated to the number of previous abortions and stillbirths" (178). The authors suggested that the occurrence of such antibodies in cord sera might represent an autoimmune-like response to p30-related antigen associated with syncytiotrophoblasts. In a separate study, Suni et al. (177) used a synthetic undecapeptide to elicit antibodies to a *gag* sequence of a HERV designated ERV-1. The undecapeptide sequence was inferred from the nucleotide sequence of ERV-1. It had 60% homology with BaEV p30 and 70% homology with Moloney MuLV p30. Using immunohistochemical assays, these authors detected cross-reactive antigens in 17 of 17 healthy human placentas at 5 to 15 weeks but not in any of 21 placentas at 16 to 41 weeks. Blighted ova (10 of 10) and choriocarcinomas (10 of 10) also were positive. Normal embryonic and adult tissues were negative. By the use of immunoblotting assays, these investigators detected a 75,000-M_r polypeptide in cultured choriocarcinoma cells and in isolated placental syncytiotrophoblasts; i.e., the 75,000-M_r polypeptide may have represented a HERV-encoded *gag* precursor or *gag-pol* fusion protein. Since the HERV ERV-1 lacked a 5′ LTR, the authors suggested that a cellular promoter may have been used for the synthesis of the detected polypeptide. Such a compensatory mechanism has been utilized by some defective avian endogenous retroviruses (74). By means of immunofluorescence microscopy, Maeda and coworkers (114) detected a protein antigen in healthy human placenta at term that cross-reacted with p30 of simian sarcoma-associated virus and gibbon ape leukemia virus. In an extension of these findings, Jerabek and coworkers (82) reported that normal-term placentas contained a protein antigen of ca. 30,000 Da "physiochemically similar to reference murine and primate type C retrovirus p30s and antigenically cross-reactive with p30 of the simian sarcoma-associated virus and gibbon ape leukemia virus primate retrovirus group." In a subsequent study, Maeda et al. (114) reported that sera obtained from patients with acute leukemia and malignant lymphoma contained an immunoglobulin M antibody that reacted with placental syncytiotrophoblasts. It occurred less frequently in the sera of pregnant women and healthy controls. The main reactive antigen was reported to be a nonglycosylated 32-Da protein that was antigenically related to simian sarcoma-associated virus p30 (114).

The studies of Kurth et al. (97) revealed a very interesting pattern of antibodies to type C viral antigens in patients with testicular teratocarcinomas. Radioimmune assays revealed that those patients with differentiated teratocarcinomas reacted more often and with higher titers than healthy controls. Antibodies to the gp70 of simian sarcoma-associated virus were found. Approximately two-thirds of all testicular tumor patients, and essentially all patients with untreated teratocarcinomas, had antibodies to p30 of this virus. Only low titers to p15E of Friend MuLV could be found. However, some sera from healthy subjects also had antibodies to p30 of simian sarcoma-associated virus.

The results summarized above provide clear evidence that the type C virus particles and HERVs that occur in placental tissues and teratocarcinomas make up at least two large diverse multicopy families that have a long evolutionary association with the human genome since they are present as multicopies in both OWM and NWM. The detection of antigens in syncytiotrophoblasts and teratocarcinomas that are related to p30 of BaEV and p30 of simian sarcoma-associated virus has been documented repeatedly. Whether the antigens detected are encoded by members of the HERV-H or the HERV-K family is uncertain. The possibility that expression occurred because of complementation events among members of these two large multicopy families remains interesting. The occurrence of antibodies to such antigens in human sera has been a regular finding possibly reflecting an immune response to highly conserved interspecies antigens. The detection of a p30 protein or 75,000-M_r protein in syncytiotrophoblasts lends credence to the belief that HERV expression occurred at the protein level. Whether such antigens elicit autoimmune reactivities warrants careful documentation. The finding (178) that a positive correlation existed between serum antibodies to p30 antigens in syncytiotrophoblasts related to simian sarcoma-associated virus p30 and complications in pregnancies warrants confirmation and extension.

HERVs AND AUTOIMMUNE DISEASE

The possibility that HERVs play an etiologic role in the autoimmune diseases (rheumatoid arthritis, lupus erythematosus, Sjögren's syndrome, mixed connective tissue diseases, and inflammatory neurologic diseases) has been discussed (1, 94, 159, 160, 180, 181, 191). Venables and Brookes have thoughtfully considered (191) how proving an etiologic association between HERVs and any one of these clinical entities is rather a daunting task. The proof involves showing that a specific HERV gene product elicits autoimmune lesions in a given target tissue or cell type while excluding already known autoimmune antigens such as the ribonucleoprotein autoantigens (Ro/SSA and La/SSB), topoisomerase I, the Ki antigen, and the like. A further complication results from the common finding, discussed above, that human sera often contain antibodies to highly conserved retrovirus interspecies antigens. The occurrence of heterophile antibodies that cross-react with such antigens greatly complicates the interpretation of results. The mechanisms responsible for eliciting autoimmune reactivities undoubtedly fall within well-established boundaries. They include molecular mimicry, as discussed recently by Horsfall (72); the nonspecific activation of effector T and B cells by retrovirus, mycoplasma, or other superantigens (2, 75); and the breaking of tolerance because of sequence homologies between retrovirus and host tissue antigens or by immune complex mechanisms (139).

That HERVs may be expressed as proteins in vivo appears not to be as improbable as most authors suggest. As documented above, there is a large body of evidence indicating that HERVs or their gene products are expressed not infrequently in vivo. For example, type C particles are relatively common in human placenta and teratocarcinomas but occur less frequently in embryonic tissues. In classic experiments, Boller et al. (15) documented that *gag*-specific anti-HERV-K antiserum reacted specifically with such type C particles, recognized a major core protein of ca. 30 kDa, and reacted specifically with the membranes of virus-producing cells. As noted above, Suni

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et al. (178) identified a p30-related antigen in normal placenta, choriocarcinomas, and blighted ova. In related studies, Suni et al. (177) identified a 75,000-M_r polypeptide in cultured choriocarcinoma cells and in the cytoplasm of syncytiotrophoblasts presumed to have been encoded by HERVs present in such cells. Jerabek et al. (82) reported the occurrence of primate type C retrovirus-related p30 protein in normal human placentas. From these results, it is clear that HERVs or HERV gene sequences are expressed in vivo. The question now becomes whether such expression is confined largely to specific cell types in the placenta or teratocarcinomas or whether such antigens participate in eliciting autoimmune diseases. The following discussion addresses this question.

One of the hallmarks of SLE is a glomerulonephritis that results from the deposition of immune complexes in the kidney. The pathogenesis of the disease has been studied in three experimental models in mice (186). For unknown reasons, retrovirus gene products, and possibly immune complexes, have been found in patients with hematologic disorders, particularly the myelomonocytic leukemias. Jacquemin et al. (80) purified a 74-kDa glycoprotein from the plasma of a patient with chronic myelogenous leukemia in blast crisis. However, this antigen also was found in patients with other blood or bone marrow dyscrasias and, to a lesser degree, in healthy individuals. By the use of highly specific monoclonal antisera, these authors found that the purified molecule had antigenic determinants in common with p15E and RT. There was no direct evidence showing that the protein was a HERV gene product, although that appeared to be the case. In a similar study, Hehlmann and his associates were able to demonstrate (68) that sera from patients with myelogenous leukemia contained an antigen (M_r, 30,000) that cross-reacted with the p30 core proteins of SSV and BaEV. The cross-reactive antigen was prominent in patients with acute leukemias or in blast crisis. Nonleukemic sera did not contain such an antigen. These studies were done before HIV-1 or HTLV-1 infections were common and presumably represented the expression of HERVs, although no direct proof of this was provided. Derks et al. (42) made analogous findings in their studies of a single patient with chronic myeloid leukemia. They were able to show that patient donor cells actively synthesized a p30 protein that cross-reacted with antiserum to SSV p30. Such cells also had high levels of RT activity. Again, no formal proof linking such activities to HERV expression was obtained. The very interesting study by Hehlmann and his associates (67) reported that sera from leukemic patients (mostly some form of myelomonocytic leukemia) contained antigens and immune complexes related to gp70 of SSV and gibbon ape leukemia virus. Some nonleukemic sera also contained such complexes. The occurrence of such antigens or immune complexes in leukemic patients correlated with an adverse prognosis. The authors speculated that HERV expression may have been the origin of such antigens.

There have been classic studies documenting the deposition of retrovirus antibody-antigen immune complexes in the kidney in association with glomerulonephritis. In SLE, the human endogenous retrovirus HEL 12, derived from lung fibroblasts grown in vitro (145), was used by Panem et al. (144) to analyze the immune complexes in the kidneys of patients with lupus nephropathy. Antiserum prepared to HEL 12 virus reacted specifically with HEL 12 virus. It also cross-reacted with SSV, BaEV, and RD114. Immune complexes were eluted from nephrotic kidneys. Fractions with a molecular weight of >200,000 contained viral antigens and immunoglobulins. After fractionation, immunoglobulins reacted specifically with HEL 12-infected cells. The kidney eluate containing antigen absorbed anti-HEL 12 activity from the anti-HEL 12 sera. However, antinuclear antibody also was found in eluates. These authors (144) suggest that "activation or *de novo* infection with C-type virus and the concomitant production of antiviral antibody result in complex-trapping glomerulonephritis." They point out that their results are in general accord with those found in studies of lupus in the NZB/w mouse model.

Mellors and Mellors (122) have carried out comprehensive studies of the occurrence of retrovirus antigen-antibody immune complexes in the kidneys of patients with glomerulonephritis associated with SLE. They reported that such immune complexes were composed of antibodies to the core proteins of type C retroviruses (RD114 and Rauscher MuLV) but not to antigens of MMTV or the avian type C viruses. Again, the classic technique of eluting antigen-antibody complexes from nephrotic kidneys was employed. Immunoglobulins in such eluates reacted with the core antigens of type C mammalian viruses but not with comparable antigens of MMTV or type C avian viruses. These authors were careful to point out the pitfalls in the interpretation of their results given the fact that SLE is a complex multifactorial disease. Among other considerations, they invoke the occurrence of subinfectious virus expression or infection by unknown human retroviruses. Again, these studies were done before infection by HIV-1 or HTLV-1 was commonplace.

Blomberg et al. (14) carried out careful studies to determine whether a correlation existed between the occurrence of SLE and antibodies to retrovirus antigens in the sera of SLE patients. They recognized at the outset that normal human sera contain antibodies to the interspecies capsid antigens of type C mammalian retroviruses and that the detection of antibodies in sera might represent a response to either endogenous or exogenous retroviruses or cross-reactivities related to antigenic mimicry. They postulated, nonetheless, that the occurrence of such antibodies served as indirect evidence for the immune response to expression of HERV proteins in vivo. On the whole, SLE sera reacted more strongly with BaEV and MuLV capsid antigens than did sera from control patients. Whole BaEV antigen reactivities were the most clearly overrepresented antiretroviral reactivities found among SLE patients. Such reactivities were greatest in SLE sera that were antiribonucleoprotein and anti-Smith autoantigen positive. A rather extensive antibody study using a spectrum of synthetic peptides homologous to conserved epitopes of *gag* and *env* of MuLV, BaEV, simian sarcoma-associated virus, Mason-Pfizer monkey virus, MMTV, HTLV-1, IAP, and HERV 4.1 was carried out. The rather surprising result was that antibodies to such synthetic peptides were as common in healthy controls as in various SLE patient subgroups. Blomberg et al. (14) concluded that "antibodies to some retroviral antigens, primarily from type C viruses, were more common in SLE patients [and] are compatible with an exposure to cross-reactive epitopes from more or less complete type C-like retrovirus(es) in SLE." Thus, these authors recognized the importance of retrovirus antigen expression but did not invoke either an endogenous or an exogenous origin.

Talal and his associates (181) also determined whether patients with SLE had antibodies to retrovirus antigens. These authors found that 22 of 61 SLE patients, and 19 of 100 patients with systemic sclerosis, had antibody to p24 *gag* of HIV-1. Such antibodies were not found in patients with adult rheumatoid arthritis, polymyositis, or chronic fatigue syndrome. Of the positive SLE patients, eight had antibody to the Sn nucleoprotein. These authors reported (180) that the proline-rich epitope shared by Sn and p24 of HIV-1 represented

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an instance in which molecular mimicry caused the elicitation of antibodies cross-reactive with a retrovirus antigen.

Studies of Sjögren's syndrome also have provided evidence for HERV gene expression in vivo and that such gene products may participate in the etiology of this autoimmune disease. As noted above (57), an IAP appears to be associated with salivary gland tissue obtained from patients with Sjögren's syndrome. Shattles et al. (170) used a monoclonal antibody to the p19 group-specific *gag* antigen of HTLV-1 to determine whether related antigens were detectable in labial salivary gland tissue sections obtained from Sjögren's syndrome patients. Thirty-one of 39 patients with primary Sjögren's syndrome were positive. However, patients with other autoimmune diseases also were positive. The antigen was absent from salivary glands of healthy controls. Remarkably, none of the positive patients had antibodies to HTLV-1 in their sera. When peripheral blood monocytes, and some established lines of epitheloid cells, were treated with phytohemagglutinin or gamma interferon, the expression of antigen was increased. The authors suggested that the detected antigen was an expressed HERV antigen possibly related to HRES-1. These authors made the interesting suggestion that the inflammatory response in Sjögren's syndrome patients enhanced antigen expression because of local cytokine secretion (170).

Banki et al. (7) carried out studies to determine whether the HERV designated HRES-1 elicited an antibody response in patients with various autoimmune diseases. HRES-1 is severely truncated but has a functional 5′ LTR and a *gag* sequence containing two LTRs capable of encoding a protein. Two *gag*-related peptides were synthesized to test for antibody response, viz., peptide 14-24 with homology to HTLV-1 *gag* p19 and peptide 117-127 with homology to *gag* p24 of HTLV-1. Donors were screened for HRES-1 antibody, using a combined cutoff level for both peptides. The authors reported the positive findings as follows: multiple sclerosis, 29%; progressive systemic sclerosis, 23%; SLE, 21%; Sjögren's syndrome, 10%; essential cryoglobulinemia, 5%; AIDS, 0%; normal blood donors, 1.5%. Considered together, these results may suggest that patients with various autoimmune diseases have a heightened antibody response to an HRES-1-encoded protein.

If HRES-1 is related to HTLV-1, one might expect it to be associated with autoimmune diseases in which HTLV-1 is considered to play an etiologic role. For example, Sato et al. (167) reported an association between HTLV-1 infection and chronic inflammatory arthropathy, i.e., a persistent oligoarthritis and a proliferative synovitis of the large joints. Anti-HTLV-1 antibody was found in the sera and synovial fluids of all patients. Moreover, HTLV-1 DNA was found integrated into the DNA of synovial fluid and tissue cells as well. Sato et al. (167) discussed five mechanisms that might account for the described HTLV-1-associated arthropathy. These authors favored the notion that the inflammatory arthropy, as well as inflammatory disease in multiple organ systems, was a consequence of "immunoregulatory disturbances caused by HTLV-1 infection." In a related study, Ziegler et al. (209) used monoclonal antisera to HTLV-1 p19 and p24 to detect related antigens in the synovial joints of patients with rheumatoid arthritis. In a study of 23 patients, approximately 45% of synovial tissues obtained from patients with early destructive rheumatoid arthritis were positive for HTLV-1 p19 and p24 antigens; i.e., antigens related to or cross-reactive with HTLV-1 p19 and p24 were expressed. Remarkably, the sera from such patients lacked antibodies to HTLV-1. In a similar study, Ziegler and Thomas (210) used monoclonal antisera to p17 and p24 of HIV-1 to detect antigens in synovial specimens obtained from patients with rheumatoid arthritis. Approximately 55% of 33 patients had p17 and p24 antigens in rheumatoid synovial tissues. Sera from these patients also were negative for antibodies to such antigens. Among other possibilities, these results, like those described for HTLV-1, suggest a compartmentalization of antigens or a compartmentalization of the immune response to them. These results therefore resemble the compartmentalization of the immune response to HIV-1 as we reported recently (190).

The hydrophobic transmembrane protein p15E encoded by the *env* gene of feline leukemia virus and MuLV is cleaved from an *env* gene-encoded precursor polypeptide, is ca. 19,000 Da in size, and is immunosuppressive. The transmembrane protein of HTLV-1 and HTLV-2 (gp21E) shares some sequence homologies with p15E of feline leukemia virus and MuLV (29). On the basis of amino acid homologies, Cianciolo et al. (29) synthesized a peptide (CKS 17) containing 17 amino acids that had significant homologies to HTLV-1 and HTLV-2 gp21E, MuLV and feline leukemia virus p15E, and HERV 4.1 p15E. Therefore, it was of interest to consider whether HERVs possessing such homologies could encode and express p15E-like proteins in vivo. Two approaches were used: the detection of p15E-like factors (173) associated with various autoimmune and malignant diseases, and the detection of serum antibodies to p15E-like factors in appropriate patient groups. Thus, Foulds et al. (51) analyzed the expression of a p15E-related epitope in colorectal and gastric cancers. These investigators found that mRNA for the suppressive epitope of p15E occurred in most human colorectal and gastric cancers. These authors postulated that p15E-related materials were elaborated by the human tumors or by the antitumor inflammatory response. In a very interesting investigation, Stöger et al. (175) studied the expression of p15E-like factors in the serum and urine of patients with malignant and benign breast tumors. Low-molecular-weight fractions in serum and urine were tested for p15E activity by the monocyte polarization technique. The specificity of p15E-like activity was determined by absorbing the test preparations with monoclonal antibodies to p15E. The patient groups consisted of 30 patients with breast cancer, 29 patients with benign breast masses, and 28 healthy controls. Patients with breast cancer were tested before and after surgical removal of tumors. Overall, the results indicated that p15E-like factors were present in the serum and urine of 77% of breast cancer patients but in only 10% of subjects with benign breast masses. The persistence of p15E-like factors appeared to correlate with the persistence of residual disease. The occurrence of p15E-like activity in the urine correlated well with serum activity. The authors were careful to point out that p15E-like factors also occur in disease states unrelated to malignancy such as chronic purulent rhinosinusitis, Graves disease, and type I diabetes mellitus. Since the occurrence of p15E-like factors decreased after surgical removal of tumors, the authors suggested that the measurement of such factors might be of value in assessing the effectiveness of therapeutic modalities. However, no direct evidence indicating that p15E-like factor expression correlated with HERV activation or expression was presented.

Evidence for the expression in vivo of the outer membrane proteins encoded by the *env* gene of HERV-K10 was reported by Vogetseder et al. (193). Three HERV-K10 recombinant envelope constructs (ENV-1, ENV-2, and ENV-6) were used for antibody assays in the following patient groups: 175 healthy blood donors; 60 patients with breast cancer; 46 patients positive for HIV; and 35 patients infected with cytomegalovirus. The positive results for antibody to HERV-K ENV constructs ranged from 11.7 to 17%. The frequencies of antibody to ENV-1, ENV-2, and ENV-3 were essentially the same. Only

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patients with elevated serum neopterin levels were positive for antibody more frequently (21% of 175 patients). When antibodies to HERV-ENV-1 were purified from positive sera, they did not cross-react with commercial HIV-1 antigen preparations. The authors state (193): "our findings conclusively establish the hypothesis that HERV-K genomes are expressed not only at the RNA level, but also at the protein level and that humans are not tolerant toward HERV-K epitopes, as we could detect antibodies against HERV-K outer membrane envelope."

In their studies of patients with autoimmune connective tissue disease, Query and Keene (156) found that such patients had antibodies that cross-reacted with p30 *gag* proteins of mammalian type C retroviruses. These authors also showed that a 70-kDa protein associated with U1 RNA cross-reacted with such antibodies; i.e., affinity-purified antibody obtained from patients with autoimmune connective tissue disease reacted specifically with a cross-reactive epitope shared by the 70-kDa protein and *gag* of many of the type C retroviruses. No cross-reactivity to HTLV-1, HTLV-2, or HIV-1 was demonstrable. These findings appear to represent a clear example of molecular mimicry. Whether the sensitizing *gag* antigen originated from activation of HERV genes or from exogenous retrovirus infection was not established. However, molecular mimicry alone could account for the observed results.

To summarize, we have addressed the central question of whether HERVs can be expressed in vivo and, as a consequence, possibly be implicated in various disease entities. As noted above, the evidence that HERV antigens are expressed in placentas and teratocarcinomas is not ambiguous. Specific HERV-related antigens have been demonstrated in syncytiotrophoblasts but not in precursor cytotrophoblasts. However, as the latter are induced to differentiate into the former by steroid hormones, there is a corresponding increase in placental type C particles and retrovirus interspecies antigens. In general, serologic studies of pregnant females disclose an increase in serum antibodies to such interspecies antigens during the first to second trimesters of pregnancy, with a decrease of such antibodies after parturition. Moreover, immunoblotting and fractionation techniques have directly detected retrovirus interspecies antigens, presumably encoded by HERVs, in placental tissues. These results are not ambiguous either.

COFACTORS IN HERV EXPRESSION

Sera from healthy individuals often contain antibodies to the interspecies antigens of mammalian type C retroviruses. Many hypotheses have been proposed to account for their occurrence: the ubiquitous subclinical infection of human populations by currently unrecognized retroviruses; the expression of endogenous tissue antigens that mimic retrovirus antigens; the derepression of genes that code for cross-reactive antigens; the expression of HERVs or HERV genes; the transactivation of HERVs or HERV genes by superinfecting viruses or other infectious agents; the activation of T cells so that the expression of endogenous retroviruses is increased; and the occurrence of cross-reactive nonspecific antibodies. HERV or HERV gene expression need not be constitutive, i.e., just sufficient to elicit serum or cell-mediated immune responses to mammalian type C interspecies antigens. On a stochastic basis, the multicopy HERVs with the most intact and least mutated genomes presumably would be those expressed most frequently. As noted above, such classes of HERVs often share homologies with BaEV and SSV. Of the various mechanisms that could cause such expression, it seems not unlikely that the inflammatory response could be the most common and important. This hypothesis is based on the observation that the inflammatory response appears to play such a preeminent role in the rheumatoid and connective tissue diseases of putative autoimmune etiology, as well as the inflammatory diseases of the central nervous system of suspected viral etiology. As a corollary, Quinn et al. (157) have reported that patients whose immune systems are in the chronically activated state have heightened susceptibility to HIV infection and progression of disease. During the normal human life span, it is not unreasonable to assume that HERVs or HERV genes could be activated numerous times, possibly by unrelated mechanisms.

Cellular Transcription Factors

Nabel and Baltimore (132) and Norcross et al. (136) outlined how T-cell mitogens may enhance or activate retrovirus replication. Thus, a core enhancer sequence, GGGACTTTCC, is found 5′ to the three Sp1 binding sites and TATA sequence of the 5′ LTR of HIV-1 (Fig. 5). The same sequence also is found in the kappa immunoglobulin enhancer and in simian virus 40, cytomegalovirus, and other DNA viruses. When T cells latently infected with HIV-1 are activated by stimulation with phytohemagglutinin or phorbol myristate acetate, HIV-1 production is stimulated about 50-fold. The activation of such T cells correlates with the induction of NF-κβ that complexes specifically to the described enhancer. The activation of the HIV-1 promoter by the cellular transcription factor Sp1 (85) is considerably more complex. For example, LTR sequences that are recognized by Sp1 are regulated by various other mechanisms, such as the simian virus 40 early promoter, the thymidine kinase gene product of herpes simplex virus, or other upstream regulatory elements. Again, these mechanisms appear not to have been exploited adequately in combination therapeutic approaches in AIDS or in autoimmune or neurologic diseases that have a multifactorial etiology.

Cytokines

Cytokines may affect retrovirus replication and gene expression in various ways. For example, mononuclear phagocytes are a major target of HIV infection. It is not unexpected that HIV production by such cells is cytokine sensitive. Koyanagi et al. (91) reported that granulocyte macrophage-stimulating factor, macrophage-stimulating factor, and IL-3 markedly increased the production of HIV in monocyte cultures. Gamma interferon either increased or decreased HIV-1 replication depending on when it was added to cultures. Koyanagi et al. (91) discussed the important implications of their findings in the pathogenesis of AIDS, particularly as they relate to therapeutic strategies.

The *trans* activation of cytokine expression was documented by Tendler et al. (185) in their studies of HTLV-1-associated myelopathy-tropical spastic paraparesis. Patients with this condition characteristically have highly activated T cells and monocytes. It is reasonable to assume that the immune activation of such cells is related to the inflammatory lesions seen in the central nervous system of such patients. The px gene of HTLV-1 encodes the transcriptional activator Tax, which plays an important role in HTLV-1 gene expression acting in *trans*. It also activates various cellular genes, including those which code for IL-2 and its corresponding receptor. Tendler et al. (185) postulated that the px gene product (transactivator) causes a constitutive production of IL-2 and its receptor, thus leading to polyclonal T-cell activation; i.e., the activated cellular immune state in HTLV-1-associated myelopathy-tropical spastic paraparesis is virally driven by the upregulation of IL-2 and its corresponding receptor. In another example of trans-

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activation, Tadmori et al. (179) reported that tumor necrosis factor alpha activated HIV expression in human monocytes. Activation appeared to be mediated by the induction of a cellular transcriptase factor similar to NF-κβ, i.e., a factor acting at the level of promoter-enhancer sequences of the HIV LTR.

Clerici and Shearer (31) reviewed the possible effects of cytokines on HIV-1 infection, particularly as they may affect the pathogenesis of AIDS. Folks et al. (50) focused their discussion on the effects of cytokines on HIV-1 expression. A detailed discussion (164) of the effects of cytokines on HIV-1 gene expression is beyond the scope of the current review. However, since it is well established that cytokines in general can effect retrovirus gene expression, it seems reasonable that they may also regulate HERV gene expression as well. Some strategies have been developed to exploit the cytokines in therapeutic approaches in AIDS (30).

Transactivation of HERVs by DNA Viruses

HIV-1 gene expression, regulated by the HIV-1 LTR, can be transactivated by regulatory genes of many of the common DNA viruses that infect humans. In this regard, and because of their possible relevance to the pathogenesis of AIDS, herpes simplex viruses types 1 and 2, cytomegalovirus, human herpesvirus 6, Epstein-Barr virus, and hepatitis B virus are of particular interest. The background literature has been reviewed by Mosca et al. (126), Gendelman and associates (59), and Barry and coworkers (9). Since it is documented (134) that cytomegalovirus and HIV-1 coinfect brain cells in patients with AIDS, it seems probable that lymphoid cells could be similarly coinfected by HIV-1, human herpesvirus 6, and Epstein-Barr virus. At the risk of oversimplification, it appears that transactivation of HIV-1 by DNA viruses occurs at the level of the LTR, involves the basal promoter and enhancer sequences, and probably involves the participation of cellular transcription factors as well. This important but complex literature warrants separate review and appraisal. Nevertheless, since it is the case that the common DNA virus regulatory gene products activate the HIV-1 LTR and HIV-1 gene expression, there appears to be no reason, a priori, why they might not similarly activate HERV or HERV gene expression.

To summarize, we favor the hypothesis that the intermittent or spasmodic expression of HERVs could account for the common occurrence in human sera of antibodies to mammalian type C interspecies retrovirus antigens. Using HIV-1 as a paradigm, we outlined above how HERVs could be activated by reasonably well-recognized mechanisms. Other mechanisms could be invoked as well. For example, the rescue of HERV sequences from human cells by the clever use of helper viruses, as described by Závada et al. (207), or by expanding the cell host range of HERV genomes by the construction of phenotypic mixes with known endogenous retroviruses, as described by Lusso et al. (112), serve as examples of alternative approaches to HERV detection and assay that may have clinical laboratory application.

MODEL FOR HERV EXPRESSION

We developed a model (130, 131) in mice that permits an analysis of how endogenous retroviruses expressed in vivo can interact with cofactors to yield a new disease entity, viz., paralytic motorneuron disease. The major interactive elements are as follows. Mice must carry multiple copies of ecotropic endogenous retroviruses, and they must be genetically permissive (150) for ecotropic retrovirus expression, e.g., $Fv\text{-}1^{n/n}$. They must be immunosuppressed either by aging or from treatment with cyclophosphamide or whole-body X-irradiation. Susceptibility correlates with a loss of $CD4^+$ T cells. Moreover, resistance to disease can be restored in cyclophosphamide-treated or X-irradiated mice by $CD4^+$ T cells. The prototype mouse strain used for such studies was the C58/wm high-leukemia strain. Paralytic disease does not occur in this strain unless it is infected with appropriate strains of lactic dehydrogenase virus. In the C58 strain, the onset of susceptibility is at about 6 months, and susceptibility increases at essentially a linear rate thereafter. We referred to the model as an example of interactive infection because neither agent alone is capable of eliciting the described paralytic motor neuron disease. AIDS, of course, is an even more dramatic example of an interactive infection.

Contag and Plagemann (33) have carried out elegant studies at the molecular level detailing the tissue and cell specificity of the described motor neuron disease. Lactate dehydrogenase virus replication in the central nervous system after peripheral injection is confined largely to the cord and its large motor neurons; i.e., replication in the brain is minimal. These investigators made the extraordinary finding that age or pretreatment of mice with cyclophosphamide increased retrovirus expression in the cord and was most prominent in motor neutrons but did not occur in young mice. This report is the first example of drug induction of endogenous retrovirus expression in a specific tissue and cell type in vivo. Perhaps the age specificity of such induction correlates with an age-dependent relaxation of endogenous virus expression, as reported earlier by Ono and Cutler (142).

We believe (189) that the value of the model lies in the fact that it provides an intellectual framework for understanding how complex multifactorial diseases may occur: i.e., the nature of the participating interactive elements; the positive and negative role of the immune response; and genetic determinants of susceptibility. In addition, it provides a design platform for diagnostic and therapeutic products. There is ample reason to believe that these orders of complexity prevail in the multifactorial etiologies of autoimmune diseases of humans and in AIDS. One would seek to determine whether HERV families are etiologic participants and how they are involved.

DISCUSSION

This review has been limited primarily to the nature of HERVs and their putative relationship to classical endogenous retroviruses, the occurrence of HERVs in tissues and cells, and evidence indicating that HERV expression at the protein level may implicate HERVs in human disease. The literature on HERVs is unusually diverse and originates from very disparate fields, thus making a review difficult. One consequence of this is that the term HERV has been used rather broadly to include repetitive DNA sequences that may or may not be related to classical endogenous retroviruses. In this review, we carefully defined endogenous retroviruses and largely confined our discussion to this subject area. We did not distinguish retrotransposons from endogenous retroviruses since there are no characteristics that distinguish one from the other. By contrast, we encountered substantial difficulty in deciding which of the severely truncated retrotransposons reasonably could be excluded in a discussion of HERVs. We elected to exclude retroposons and simpler structures from consideration since they fell more within the province of repetitive DNA sequences that lack LTRs. The occurrence of LTRs in endogenous retroviruses is a point of major significance because LTRs play a preeminent role in gene expression. One unfortunate conse-

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quence of this decision was that we did not include in this review a discussion of such interesting retroposons as S71 (198) and HRES (7). Reviews of such interesting repetitive DNA sequences have been published recently (25, 44, 56, 76, 102, 201).

Any attempt to group HERVs into natural or related categories is confronted with a number of difficulties. In order of importance, they are the lack of a sufficient base of hard experimental data, bias introduced into experimental methodology by the kinds of probes used for detecting DNA sequences or the primers used for PCR amplifications, and bias resulting from the perspective and field of specialization of a given investigator. The following examples help to illustrate these points. When highly conserved *pol* or *gag* sequences are used as probes, it is not surprising that they detect corresponding highly conserved DNA sequences in test DNA libraries. This can cause a bias concerning the possible diversity of detectable HERVs; i.e., it can yield a constrained portrayal of the diversity of HERVs that may exist. As another example, Doolittle et al. (44) made the cogent point that "mainstream metabolic enzymes have been preserved to the extent that prokaryotic and eukaryotic versions still are 50% identical some two billion years after their diversion." The highly conserved *pol*-encoded enzymes appear to conform to this dictum. Thus, Chiu et al. (28) have traced the evolution of *pol* gene progenitors for oncogenic retroviruses. This does not mean, however, as stated by Callahan (22), that there are two families of human endogenous retroviral genomes, i.e., class I mammalian type C-related sequences and class II A, B, and D virus-related sequences. Such a statement reflects a bias of a field of specialization that fails to consider large bodies of evidence to the contrary. For example, HTLV-1 surely is different from HIV-1 in a large number of parameters, although they are both class C mammalian retroviruses. Similarly, class II MMTV is vastly different from IAP.

An equivalent conceptual difficulty derives from the proposal of Kröger and Horak (95) and Larsson et al. (100) that HERVs be classified according to the amino acid that complexes with the tRNA homologous to its primer binding site. There are at least two shortcomings in this proposed scheme of classification. The 18-nucleotide primer binding site on HERV DNAs and the corresponding tRNA sequences probably do not adequately represent the molecular or biological properties of a full-length provirus 8 to 9 kb in size. For example, proline binds to the tRNA of HTLV-1 and MuLV. Nevertheless, it is safe to say that these entities are vastly different.

Another approach to categorizing HERVs depends on making comparisons of sequence homologies of putative retroviruslike genes of a given HERV with others or their deduced amino acid sequences and then computing percent homologies. Unless the basis for making such comparisons is authenticated by other objective evidence, such categorizations can result in misleading conclusions. For example, it is often stated in the literature that the nucleotide sequence of HERV-K10 (141) is related to the MMTV genome; i.e., the implication is one of a significant similarity. Ono et al. (141) precisely showed that a comparison of the deduced amino acid sequences encoded by the *pol* regions [of HERV-K10] indicated that this provirus is closely related to IAP-H18 (type A retrovirus), a squirrel monkey retrovirus (type D retrovirus) linked to the simian acquired immune deficiency syndrome, and even more so to MMTV (type B retrovirus). Larsson's statement (100) that HERV-K is a mosaic-type endogenous provirus accurately reflects the experimental facts. In reading the HERV literature, one must be quite careful to ascertain whether purported relationships among HERVs are supported by substantial experimental data. As noted above, one of the major limitations in categorizing HERVs is the lack of such data. Given the current status of the field, we believe that the main parameters of interest are whether HERV genomes are full length, whether they are multicopy or not, whether they are type C, B, or A entities on the basis of reliable electron microscopy and molecular data, and the mechanisms that regulate their expression. If HERVs are full-length genomes and occur as multicopies, the implication is that complementation or transactivation could more likely play a significant role in their expression, i.e., the stochastic concept of expression. The challenge to clinical laboratories is to detect and quantify HERV gene expression in various clinical settings.

Since antibodies in human sera often are cross-reactive with retrovirus interspecies antigens and are widely distributed in human populations, an explanation is needed for their occurrence. We have suggested that such antibodies may represent an immune response to products of HERV genes highly conserved in the human genome; i.e., such genes may be expressed spasmodically under the circumstances discussed above. Their expression also could elicit autoimmune inflammatory responses. For example, it is well established clinically that respiratory viral infection can be a very inflammatory event in an allergic individual and thus serve as a HERV-activating event. A spasmodic triggering mechanism would be in accord with the protracted course of autoimmune reactivities and predisposing genetic mechanisms. The spasmodic activation of one or more families of HERVs could well suffice.

The elegant studies of Shih and his associates (171) provide insight into the kinds of HERV families that may be implicated. In their analyses, Shih et al. (172) exploited the principle that if a given proviral DNA is inserted at the same (DNA) site in two species, it undoubtedly was present in their common ancestor. Their results showed, for example, that proviral HERV-E (4.1) is located in at least one identical site in humans, chimpanzees, gorillas, orangutans, and baboons. HERV-R(3) is located in at least one identical site in humans, chimpanzees, gorillas, orangutans, and baboons. These results indicate that the same site was occupied by these HERVs in a common ancestor of OWM and apes. We consider such HERVs to be primordial, i.e., that they were in the primate germ line well before OWM and apes diverged about 30 million years ago. HERV-E (4.1) is one member of a multicopy 50-member subfamily. HERV-R(3) is the single clone of a single-copy subfamily. HERV-Ia is a single member of a 20-member subfamily. Therefore, it is easy to conceive that under the appropriate circumstances complementation could occur among such HERVs; i.e., their expression could well be a stochastic event. Given these circumstances, one could regard their expression as even being an inevitable event. For convenience (more than logic), we consider the endogenous retroviruses introduced into the primate genome after the OWM-ape divergence, or the NWM-OWM split, as being contemporary HERVs in an evolutionary sense. This implies that their distribution in and randomization among primates is an ongoing process. HERVs related to HTLV-1 appear to fall into such a category. In an evolutionary sense, it appears that HIV-1 is at the preliminary stage of its evolution.

To date, the bulk of the HERV literature deals with their detection, preliminary characterization, possible evolutionary origin, and putative relatedness either to each other or to retroviruslike DNA sequences. There has been a tendency to group HERVs into families, even though the evidence for their relationships often appears to be tenuous. The studies of Ono et al. (140) discussed above illustrate this point. There is a need for the principals in the field to collectively identify HERV

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TABLE 6. Representative full-length HERVs

Original designation	Name based on amino acid bound to tRNA	Characteristics
HERV 4.1 (multicopy)	HERV-E ($tRNA^{Glu}$)	8.8 kb; ca. 50 copies per genome,[a] not tissue specific, primordial in origin, not related to other HERVs; *gag-pol* sequence homologies to BaEV; similar sequences in OWM and NWM and apes; full-length RNA transcripts found; long ORF for *env* gene
RTVL-H (multicopy)	HERV-H ($tRNA^{His}$)	Large polymorphic family mostly with truncated genomes occurring in high copy number; similar sequences found in OWM and NWM, indicating a primordial origin; *pol* shows homologies to Moloney MuLV; *gag* shows homologies to p30 of BaEV
HTDV-H (multicopy)	HERV-K ($tRNA^{Lys}$)	9.2 kb; ca. 50 copies per genome; homologies to IAPs and type D and B retroviruses; homologous sequences in OWM and NWM; full-length RNA transcript made; encodes a full-length *gag* protein and functional protease in *E. coli*; expression in cells steroid sensitive; found in placental tissues and teratocarcinoma cells; appears to be a classical defective endogenous retrovirus
HERV-K(C4) (multicopy)	HERV-K(C4) ($tRNA^{Lys}$)	6.4 kb; ca. 50 copies per genome; found primarily in intron 9 of C4A6 complement gene; sometimes found in C4B gene; homologous sequences in OWM; approximately 60–64% sequence homologies to *gag-pol* of HERV-K10+; significant *pol* homology to HERV HML-3, HML-5, and HML-6; may be principal contributor to the interlocus and interallelic heterogeneity of C4 genes
HURRS-P (multicopy)	HERV-P ($tRNA^{Pro}$)	8.1 kb; 20–40 copies per genome; primordial origin with homologous sequences found in OWM and NWM
HERV-R (single copy)	ERV-3 ($tRNA^{Arg}$)	9.9 kb; expression of RNA is cell type specific in the placenta and responsive to human chorionic gonadotropin; full-length and spliced RNA transcripts found in placenta

[a] Copies per haploid genome.

prototypes and to define how such prototypes differ or are similar. Table 6 indicates the parameters that might be considered. All of the listed HERVs are authentic proviruses with full-length genomes, differ in the amino acid bound to tRNA, are considered to be of the C type, and are of the primordial category; some express full-length RNA transcripts; the nucleotide sequence of several of them has been determined; and all of them appear to be different. One (HURRS-P) is not related to the murine retroviruses. We could not estimate which type B or A HERVs would serve as the most appropriate prototypes. Overall, the identification of the most appropriate HERV prototypes is beyond our level of expertise. There is, nonetheless, an urgency for a consensus on the principles to be applied for identifying the most appropriate HERV prototypes.

The need to establish reference prototypes for comparative and clinical laboratory studies, which is a well-established scientific principle, is evident from numerous publications in the HERV literature. For example, two groups of defective type C particles are well known: those produced by human teratocarcinoma cells in vitro (HTDVs), and those that are abundant in the placenta. In a definitive study, Löwer et al. (109) showed that cell lines (GH, Tera-1, and Tera-2) established from a human testicular teratocarcinoma produced HTDVs in vitro and that their production was inducible. In an equally definitive study, Boller et al. (15) documented that HERV-K encodes such type C virus particles. Subsequently, La Mantia et al. (99) derived a HERV from a subclone of the teratocarcinoma lines evidently established initially by Löwer et al. (109). This HERV, "in accordance to the HumanGene mapping nomenclature," was named ERV-9 and was asserted to be part of a novel human endogenous retroviral family (99). We perceive a linkage among the results of Löwer et al. (109), Boller et al. (15), and La Mantia et al. (99) and perhaps some of the HERVs studied by Mager and her associates (115, 116, 199, 200). Such findings pose several important questions: how related are the HERVs studied by these groups of investigators; which HERVs would be representative of these so-called families; and what would be the most appropriate prototypes. The development of appropriate diagnostic and therapeutic products clearly is related to the resolution of such questions.

Ono et al. (141) completely sequenced HERV-K10+ and then compared some of its sequences with those of previously reported HERVs. These authors found, for example, that a nucleotide sequence of 525 bp of HLM-2, originally described by Callahan et al. (23), corresponded to positions 5630 to 6151 of HERV-K10+; i.e., the integrase subregion of the HLM2 *pol* gene showed a homology of 88% to the corresponding HERV-K10+ sequence. This led Ono et al. (141) to postulate that HLM2 was a member of the HERV-K family. Ono et al. (141) also compared the sequences of HERV-K10+ with those of HERV HM 16, originally described by Deen and Sweet (39). The *pol* gene of HM 16 showed 89% homology to the *pol* gene of HERV-K10, thus indicating that it too was a member of the HERV-K10+ family. As noted previously, Medstrand and Blomberg (120) analyzed their HML HERVs and also found sequence homologies to HERV-K10. Brodsky et al. (19) found HERV-K *pol* gene expression in blood leukocytes from healthy donors. There are many ways to interpret these overlapping results. We simply note that such results make a very strong case for defining reference HERV prototypes so that the various HERVs isolated to date can be compared in a systematic and definitive manner. In fact, such work is essential if accurate diagnostic reagents are to be developed for HERV detection and monitoring.

Because of its extraordinary importance, we address again the dictum of whether it is the case that HERVs cannot be expressed as proteins in vivo. Throughout the HERV literature, it is stated repeatedly that HERVs cannot be expressed as proteins for three primary reasons: defects in their gene or LTR sequences; excessive numbers of stop codons in various reading frames; and excessive frameshift mutations. A priori, there is nothing wrong in the basis for these arguments, which are well founded at the molecular level. However, we extensively reviewed the evidence documenting that HERVs, in fact, very often are expressed as proteins in vivo. A partial summary is presented in Table 7.

It is important at this point to take a more global approach.

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TABLE 7. Evidence that HERV antigens are expressed in vivo

Parameter	Antigenic cross-reactivities[a]	Putative disease association	Reference(s)
Type C virus particles in:			
Placenta and oocytes	gp70 of BaEV; p30 of RD114	Complications of pregnancy	82, 83, 101, 113, 135, 143
Teratocarcinomas	HERV-K10	Teratocarcinomas	15, 20, 98, 108, 109, 168
Embryonic lung (HEL 12)	p30 of SSV and BaEV	SLE	143–145
Human embryos	RD114	Unknown	21, 125, 154
Skin lesions	Unknown	Psoriasis	78, 135
Type B virus particles in:			
Monocytes from breast cancer patients	Not determined	Unclear	3, 140
Teratocarcinoma cell line	MMTV core proteins	Unclear	48, 140
Intracisternal type A particles			
Lymphoblastoid cell line	Not determined	$CD4^+$ T-cell deficiency	62
Lymphoblastoid cell line	Not determined	Sjögren's syndrome	57
Antigen in serum or plasma			
Circulating antigen	74,000-kDa protein related to p15E of MuLV and FeLV	Hematologic disorders, particularly chronic myelogenous leukemia	68, 80
Circulating antigen	70,000-kDa protein related to p30 of BaEV and SSV	Myelomonocytic leukemia	68
Circulating antigen	p30 related to SSV	Myeloid leukemia	42
Circulating antigen-antibody complexes	gp70 related to SSV and GaLV	Myelomonocytic leukemia	67, 68
Immune complexes in kidney			
HEL 12 antigens	BaEV, GaLV, wooley monkey interspecies antigens	Glomerulonephritis in SLE patients	144
Core antigens, mammalian type C viruses	p30 of RD114 and SSAV	Glomerulonephritis in SLE patients	122
Serum retroviral antibodies			
Sera from SLE patients	Antibodies to BaEV, MuLV, SSAV, MPMV, and HTLV-1 antigens	SLE	14, 180, 181
Cord blood sera	p30 of RD114	Unknown	178
Sera from patients with hematologic malignancies	Antibody to p30 of SSAV	Lymphoid and myeloid cancers	67, 68, 80, 114
Sera from normal adults	Antibody to BaEV and SSV	None	98

[a] RD114, feline leukemia virus; GaLV, gibbon ape leukemia virus; SSAV, simian sarcoma-associated virus; FeLV, feline leukemia virus; MPMV, Mason-Pfizer monkey virus.

The question asked is whether mouse endogenous retrovirus expression in classic mouse model systems is to some reasonable degree duplicated by HERVs in humans. For simplicity, consideration is restricted to four sets of phenomena: are the type C, B, and A virus particles found in mouse tissues also found with reasonable frequency in human tissues; are the circulating retrovirus antigens found in the blood of mice also found in human blood; can the retrovirus antigen-antibody complexes found deposited in the kidneys of mice that serve as models for human SLE also be found in human SLE-associated glomerulonephitis; and are the inflammatory neurologic lesions elicited in part by mouse endogenous retroviruses also found in humans.

Type C virus particles that are classic defective endogenous retroviruses readily are found (Table 5) in human placenta, embryonic tissues, oocytes, and teratocarcinomas. Possibly they are related to human disease as discussed previously. The occurrence of type B particles in the T47D line of human breast cancer cells is well established (90, 140), as is their hormone responsiveness. Their role in disease is unknown. The occurrence of type A particles is not frequent, and their role in disease also is unknown. The occurrence of such HERVs surely requires HERV genome expression at the protein level. We discussed above the abundant evidence of an immunologic response to them.

The evidence for the occurrence of HERV antigens in human blood is well documented. We discussed above the findings of Jacquemin et al. (80), Hehlmann and his coworkers (66–68), and Derks et al. (42). In some instances, the molecular nature of the antigens was determined. For example, the 74-kDa glycoprotein studied by Jacquemin et al. (80) appeared to cross-react with HERV-encoded p15E or *pol*. The 30,000-M_r protein described by Hehlmann et al. (68) cross-reacted with p30 core proteins of SSV and BaEV. Hehlmann et al. (67) reported that sera from leukemia patients contained antigens (or immune complexes) related to gp70 of SSV and gibbon ape leukemia virus. We do not understand why such antigens were found principally in patients with some form of myelomonocytic leukemia, nor do we understand their origin. One of the most ready explanations would be that they signify infection by a hitherto unknown retrovirus or represent expression of previously undetected HERVs.

One of the most compelling pieces of evidence documenting HERV gene expression in vivo recently was published by Sauter et al. (168). These authors reported that HERV-K10 encoded a 73-kDa protein that was cleaved by HERV-K10 protease to yield proteins p22/p26, p30, and p15/16. With the Tera 1 cells described above, it was found that Tera 1 cells expressed an 80-kDa HERV-K10 *gag* polypeptide and processed proteins of 17, 19, 22, 30, and 39 kDa. More importantly, an 80-kDa protein corresponding to HERV-K10 *gag* polypeptide was found in the cytoplasm of tumor cells obtained by biopsy. Using a recombinant HERV-K10 *gag* antigen, these authors detected antibodies to HERV-K10 *gag* in 0.1 to 0.5% of

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healthy subjects (low titers) and in 2.0 to 4.0% of tumor patients (titers of 80 to 640), while patients with seminomas had antibody titers up to 2,560. These results constitute clear evidence for HERV gene expression in human tissues and cells and suggest diagnostic application of HERV gene products.

The analysis of viral antigen-antibody complexes in experimental models of glomerulonephritis in mouse models of SLE (186) have conformed to a classic pattern: elution of such complexes from kidney tissues followed by the specific identification of the antigens and antibodies that make up such eluates. We noted above that Panem et al. (144) analyzed the immune complexes in the kidneys of patients with lupus nephropathy. The antigens of human embryonic lung HERV HEL 12 were found. Analogous studies have been carried out by Mellors and Mellors (122). Taking a different approach, Bermas et al. (11) demonstrated that sera from mice with experimental SLE and from SLE patients reacted with recombinant HIV-1 gp120 and synthetic peptides of the HIV-1 envelope. These results established the occurrence of antibodies in human sera to explicitly defined peptides of HIV-1. Such cross-reactivity presumably can be attributed to endogenous retrovirus gene sequences expressed in both mice and humans. From the results of such findings, it is difficult to escape the conclusion that HERV expression as a protein(s) occurs in vivo.

There are three well-defined models of endogenous retrovirus-associated neurologic disease in mice: the age-dependent lower motor neuron disease that occurs naturally in feral mice (54); the neuropathogenic mutant of Moloney MuLV (203); and the endogenous retrovirus, age-dependent, dual viral infection model developed by us (130) and Contag and Plagemann (33), which they referred to as ADPM. The immunologic abnormalities that characterize ADPM may occur to some degree in multiple sclerosis and HTLV-1-associated myelopathy-tropical spastic paraparesis. However, we have been unable to find in the literature evidence for a linkage among HERV activation, protein expression, and neurologic inflammation in these two human diseases. However, we would not consider it at all extraordinary if the inflammatory exacerbations that occur in multiple sclerosis (or rheumatoid arthritis) ultimately could be linked to HERV activation.

Genetic factors that regulate HERV expression in mice are well documented, as summarized above. The role of genetic factors in the clinical expression of tropical spastic paraparesis in discrete human populations (81) and differences in survival rates in AIDS suggest an important genetic component. However, hard data on the genetics of HERV expression in humans are largely lacking.

The inability to "rescue" HERVs or otherwise demonstrate their infectivity by cocultivation techniques remains a major but not unexpected stumbling block in the field. Todaro et al. (188) accurately predicted this difficulty as a result of their exhaustive studies in attempts to rescue nondefective endogenous type C viruses from primate tissues. Endogenous type C viruses were isolated only from baboons and a closely related species; i.e., such viruses were not isolated from OWM or NWM, apes, or human tissues. For one species of baboon (*Papio papio*), only one of nine indicator lines proved to be effective in cocultivation experiments. Todaro et al. made several cogent observations (188): nondefective endogenous type C viruses appeared to be rare among certain groups of primates; primate host cell genes may be very effective in limiting productive infection in most primate species, with *Papio* and *Theropithecus* being the sole exceptions; baboons represent an unusual evolutionary "window" through which nondefective viruses may be observed; and additional approaches will be required to recover transmissible HERVs from the majority of primate species. The literature we reviewed provides some clues to approaches that may be worthwhile. The halogenated pyrimidines, steroid hormones, cytokines, human chorionic gonadotropin, and retinoic acid stimulate HERV particle production and RNA synthesis in appropriate indicator cell cultures. Possibly, this line of investigation should be expanded with more careful attention paid to dose regimens employed. In general, the demethylating agent 5-azacytidine (69) enhanced HERV RNA expression. Perhaps its application needs to be exploited. A large body of evidence indicates that established lines of fully differentiated syncytiotrophoblasts might serve as appropriate indicator (NP) cells. A corresponding baboon cell line might be even better. The rationale is that such cell lines are known to be permissive and carry multiple copies of endogenous retroviruses of different classes in their genomes such that effective complementation might occur when such lines are transfected with full-length relatively nondefective HERVs such as HERV-K10+. Such advances would have application to clinical laboratory studies.

Currently, there is an urgent need to develop diagnostic markers for the occurrence of HERVs in both health and disease. There are several guidelines indicating how this can be accomplished best. Historically, Barbacid et al. (8) and Snyder and Fleissner (174) discussed, respectively, how antibodies to modified cellular glycoproteins or to carbohydrate structures of the Forsmann type can be mistaken as antiretrovirus antibodies. Venables and Brookes (191) and Talal et al. (181) pointed out that care must be taken to distinguish antibodies to autoantigens from those that are specific for endogenous retroviruses. For example, Möstl et al. (127) reported that sera from patients with a myelodysplastic syndrome cross-reacted with HTLV-1 and HTLV-2, although there was no evidence to suggest that such patients had ever been infected by either of these viruses. One approach used to resolve these problems was to directly isolate and characterize putative antiviral antibodies or their corresponding antigens, as shown, for example, by Derks et al. (42) and Hehlmann and his associates (68). Such methodologies were required when sera were tested at low dilutions (<1:20) under circumstances in which nonspecificity was an inherent problem. A solution to these vexing problems lies in the use of explicitly defined synthetic peptide antigens. Antibodies to such antigens can be detected at higher serum dilutions (1:100 or greater), and virus-specific epitopes can be identified as the reactive antigens. Employment of such test systems requires the accumulation of data from appropriate test subjects that certify acceptable levels of sensitivity (ca. 95%) and specificity (again, ca. 95%) for the clinical laboratory tests to be carried out, i.e., accurate cutoff points. As we have pointed out (190), the occurrence of so-called indeterminate results, as in HIV-1 urine antibody tests, does not mean that the results necessarily are nonspecific. Indeed, the "specificity of the nonspecific" can be exploited to develop new fields of knowledge: again, a general principle in clinical laboratory diagnosis. Thus, such results can lead to the identification and utilization of appropriate surrogate markers in the clinical laboratory setting. Antibodies to HERV gene products are just now becoming a utilizable tool. Their application can be exploited by innovative approaches to the utilization of recombinant antigens as they become available.

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ADDENDUM IN PROOF

The effects of retroviral peptides on the balance of the immune system have been recently reviewed (S. Haraguchi, R. A. Good, and N. K. Day, Immunol. Today 16:595–603, 1995). Although the authors focused on exogenous retroviral peptides, we discuss how such findings are important to the expression of endogenous retroviruses particularly as they apply to the pathogenesis of chronic diseases characterized by an imbalance of the immune system.

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