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Replication and Pathogenesis of the Human T-Cell Leukemia/Lymphotropic Retroviruses

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A. Introduction

Human retroviruses represent an emerging class of complex pathogens involved in a wide variety of maladies, including leukemias and lymphomas, diseases of the central nervous system, and immune function impairment. These have recently been reviewed by Wong-Staal and Gallo. Four different types of human retroviruses have been isolated to date: the etiological agents of a malignant T cell leukemia/lymphoma, the virus HTLV-I which causes the disease ATLL, two viruses associated with more benign forms of T-cell leukemia (HTLV-II), and the etiological agent of the acquired immune deficiency syndrome and related disorders (HIV). Additionally, retroviruses of genomic organization similar to that of HIV but differing markedly in DNA sequence have recently been isolated among persons in West Africa (Kanki et al. 1985; Clavel et al. 1986).

As far as they have been characterized to date, the human retroviruses display interesting features of growth regulation not previously observed for the well characterized murine and avian retroviruses. The following presents a brief overview of the some of the unusual features of human leukemia viruses with some discussion of similar features in the bovine leukemia virus and simian T-cell leukemia virus, which have genomic organization similar to that of the human T-cell leukemia viruses.

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B. Pathogenesis

The T-cell leukemia and lymphoma induced by HTLV-I and -II all appear only after a very long incubation period, measured in decades (Catovsky et al. 1982). Infection is marked by seroconversion, but there is some evidence that seroconversion may occur only after very prolonged periods, ranging from 10 to 15 years from the time of infection at birth to the time of seroconversion in the teens. There is an absence of viremia in the patients and a notable lack of virus expression even in fresh tumor cell populations (Franchini et al. 1984). Stimulation of infected patient T cells with mitogens results in the expression of high levels of viral RNA and protein and the budding of virus particles (Poiesz et al. 1980).

T cells from infected patients can be made to transform normal peripheral blood T cells from uninfected people (Chen et al. 1983; Popovic et al. 1983; Miyoshi et al. 1981; Yamamoto et al. 1982). Such transformation is generally accomplished by co-cultivation and is very difficult to accomplish with cell-free virus. The transformed cells have the appearance of tumor cells, characterized both by a distinctive set of surface markers including the T4 antigen and by large lobulated nuclei similar to those of the tumor cells. The fresh tumors cells and cell lines immortalized by HTLV-I express abnormally high levels of the interleukin 2 (IL-2) surface receptor.

The absence of viremia in infected persons and the difficulty of free infection may help to explain the epidemiology of infection transmission. For most populations, including those in the Pacific rim, particularly Japan and Taiwan, and in the Caribbean, Africa, and the United States, transmission is limited to family contexts (Blattner et al. 1983). Transmission from mother to child and from infected male to female partner is documented, whereas transmission from infected female to male sex partners is thought to be rare. The virus is also transmitted by needle, either by blood transfusion or by hypodermic syringe. The latter route appears to be a significant factor in current transmission patterns of the virus, as large proportions of certain populations – for instance, intravenous drug abusers – have been found to be infected with either HTLV-I, HTLV-II, or HTLV-IV, depending upon the geographical region.

C. Genomic Organization

How might one explain the limited replication and the pathogenesis of these viruses in molecular terms?

The genomic structure of the human leukemia viruses differs from that of other retroviruses characterized to date except for the two very close relatives of these viruses, the simian T-cell leukemia virus type I (STLV-I) and, more distantly, the bovine leukemia virus (BLV). The latter, like HTLV-I, -II, and -V, is poorly infectious, and it is transmitted most commonly by the veterinarian needle. The unusual features of the organization of these viruses is pictured in Fig. 1. As with all other retroviruses the human leukemia retroviruses contain genes that encode the virion internal capsid proteins (*gag* gene proteins), genes that encode replication functions (reverse transcriptase, integrase, and protease), and genes that specify the exterior proteins which are embedded in the lipid layer that surrounds virion. The envelope protein is comprised of an exterior glycoprotein and an integral transmembrane protein. The organization of the virion structural genes and replicative genes is similar to that of the simplest avian, murine, and feline viruses.

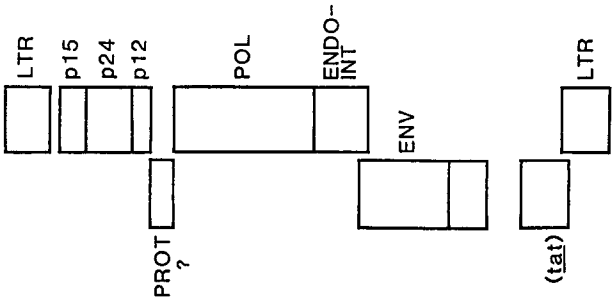

Fig. 1. Provirus structure of HTLV-I

The genome of HTLV-I, HTLV-II, and BLV viruses differs from that of other retroviruses by the presence of approximately 1500 nucleotides located between the 3′ end of the envelope glycoprotein and the 3′ LTR (long terminal repeat) (Seiki et al. 1983; Haseltine et al. 1984; Shimotohno et al. 1984). This region, called pX, has the capacity to encode multiple polypeptides of the size of 100 amino acids or greater. For an analysis of the coding capacity of the pX region of HTLV-I, see the review by Haseltine et al. (1984). Similar analyses indicate that the corresponding regions of HTLV-II and of BLV have the capacity to encode numerous polypeptides.

It has been demonstrated that the pX region of HTLV-I specifies at least three polypeptides which are made in infected, activated T cells (Kiyokawa et al. 1985). The largest of these proteins – of sizes 42 kD, 38 kD, and 36 kD for HTLV-I, II, and BLV, respectively – encode a protein that is located primarily in the nucleus (Goh et al. 1985; Slamon et al. 1985). Initially we have called this protein the *X-lor* protein for the product of the long open reading frame within the X region; however, we now refer to it as the *tat* gene product for *trans*-activator (see below) (Sodroski et al. 1985b). A subscript, tat_{I}, tat_{II}, or tat_{BLV} or tat_{STLV}, denotes the virus of origin. Approximately half the people infected with HTLV-I, whether symptomatic or not, produce antibodies to this protein. The *tat* protein is also called X or pX40 by others who have confirmed the existence of this gene product in HTLV-I and -II infected cells (Felber et al. 1986; Seiki et al. 1986).

The *tat* product is synthesized from a doubly spliced messenger RNA species

which includes transcripts of portions of the 5′ LTR, a small sequence located immediately 5′ to the envelope gene, and the distal two-thirds of the pX region through the end of the 3′ LTR (Sodroski et al. 1985b; Seiki et al. 1985; Wachsman et al. 1985; Aldovini et al. 1986).

It has recently been reported for HTLV-I that this same messenger RNA species encodes two other polypeptides from an overlapping reading frame (Fig. 1) (Kiyokawa et al. 1985; Nagashima et al. 1986). The initiating codons for the larger of these two polypeptides is located 5′ to the site of initiation of the *tat* gene product. The same splice donor-acceptor combinations as is used for production of the *tat* gene product places the alternative open reading frame in the correct register with a second open reading frame which overlaps that used to produce the *tat* gene product. The product of this second initiation event is a 27 kD protein. The protein is phosphorylated and located predominantly in the nucleus (Kiyokawa et al. 1985). The protein is called pp27, denoting both its size and the observation that it is phosphorylated. A second polypeptide is also synthesized from the same reading frame as is the pp27 protein. This third product of the pX region is thought to be initiated at an AUG codon within the second coding exon of the messenger RNA. This protein is also phosphorylated and has an apparent molecular weight of 21 kD; it is located primarily in the cytoplasm.

It is notable that the genomes of HTLV-II (Shimotohno et al. 1985), STLV-I (Watanabe et al. 1985), and BLV (Sagata et al. 1985a, b) all possess the capacity to encode similar alternative reading frame polypeptides. Indeed, there is evidence that BLV, as does HTLV-I, in fact also encodes such proteins (Yoshinaka and Oroszlan 1985). It is a curosity that these proteins do not raise antibodies in infected people. No reactivity to these smaller proteins is observed in cattle or sheep infected with BLV. The complete coding capacity of these virus has not yet been fully explored. It is conceivable that other virally encoded proteins which are of low antigenicity in infected people are present in virus-infected cells.

D. Trans-Activation: The *tat* Protein

The phenomenon of *trans*-activating retroviral gene products was first reported for HTLV-I and -II (Sodroski et al. 1985b). It was observed that the LTRs of HTLV-I and -II function much more efficiently as promoter elements in infected than in uninfected cells (Fig. 2) (Sodroski et al. 1985b). A positive *trans*-activating genetic regulatory system requires at least two elements, the *trans*-activator product and a *cis*-acting responsive element.

The *trans*-activator product of HTLV-II was initially identified as the product of the HTLV-II pX open reading frame (Sodroski et al. 1985a). It has since been reported that the pX open reading frame of HTLV-I and of BLV also encodes a *trans*-activator (Rosen et al. 1986). Isogenic cell lines which differ only in their ability to express the long open reading frame product are capable of *trans*-activation. Gene expression directed by a plasmid which carries the *trans*-activator gene has been shown to stimulate the homologous LTR of HTLV-I, -II, and BLV (Sodroski et al. 1985a; Pashkalis et al. 1986; Rosen et al. 1986; Fujisawa et al. 1986). Plasmids constructed so as to eliminate the possibility of producing the pp27 and pp21 gene products are also capable of *trans*-activation as measured in transient cotransfec-

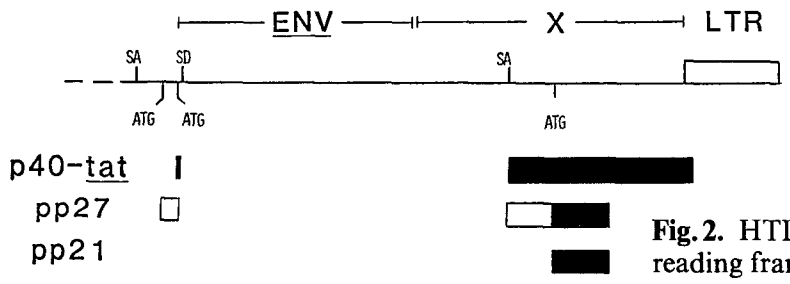

Fig. 2. HTLV-I X region illustrating the open-reading frames known to encode protein

tion assays (Kiyokawa et al. 1985). The LTR of HTLV-I can also be activated by the *tat* gene product of either HTLV-I or -II but not by the *tat* BLV product (Sodroski et al. 1985a; Rosen et al. 1986).

The increase in LTR-directed gene expression induced by the *tat* genes is accompanied by an increase in the steady-state level of corresponding messenger RNA species (Sodroski et al. 1985b; Felber et al. 1985). The increase in messenger RNA levels of heterologous genes directed by the LTR corresponds very roughly to the level of increase observed for protein expression. However, precise correspondence is difficult to document and post-transcriptional alterations in the efficienty of mRNA utilization cannot be ruled out entirely. A cautionary note is in order. At present *trans*-activation of viral genes is only inferred from the ability of the *trans*-activator proteins to increase the expression of heterologous genes directed by the HTLV LTRs. Direct induction of viral genes by the *trans*-activators has not yet been reported. Therefore the ability of the *tat* gene alone to stimulate the expression of viral genes is not established.

The *cis*-acting regulatory sequences, called TAR (*trans*-acting responsive region), were initially found to be located in the U3 region of the viral LTR, entirely 5′ to the site of initiation of viral RNA synthesis (Rosen et al. 1985). It was noted above that the U3 element of the HTLV-I and -II LTRs contained 21 nucleotide sequences repeated several times and that the sequences of these repeat units were preserved between HTLV-I and -II (Sodroski et al. 1984). It was also observed that except for these repeated sequences and for a short region near the site of RNA initiation, the sequences of the HTLV-I and -II LTRs are notably different as compared to the extent of conservation of other parts of the genomic sequences. Recently synthetic oligonucleotides which correspond to these 21-nucleotide long sequences have been demonstrated to convey a response to the *trans*-activator upon heterologous promoters (Shimotohno et al. 1986). The response to the *trans*-activator is observed when the 21-nucleotide repeat sequences are located proximal to the promoter and is irrespective of the orientation of the 21-nucleotide sequence with respect to the promoter. In some experiments a single repeat unit suffices to convey the *trans*-activation response (Rosen et al., to be published) whereas others report that two or more 21-nucleotide long sequences, tandemly repeated, are required for the *trans*-activation effect. These repeat units are called TAR-21 sequences to denote the observation that they convey a responsive phenotype (Rosen et al., to be published).

Another cautionary note is appropriate. Although the TAR-21 sequences do permit increased expression of both homologous and heterologous promoters in the presence of the *trans*-activators, the response is weak and the level of expression of heterologous genes is one or two orders of magnitude below that observed for promoters and their natural configuration – even for promoters which contain 5′ deletions that preserve only the TAR-21 sequence located proximal to the promoter (Rosen et al. 1985). This observation suggests that promoter strength and inducibility depend upon the sequences adjacent to TAR-21.

Two other curious features of the viral promoters are notable. The promoter strength of HTLV-I is dependent upon sequences located 3′ to the site of RNA initiation, within the R and U5 regions of the LTR (Derse and Casey 1986; Rosen et al., to be published). A set of nested deletions originating in the U5 region of the HTLV-I LTR and extending to the site of RNA initiation results in a progressive weakening of promoter activity. Gene expression directed by such altered LTRs is inducible by the *trans*-activator genes, although the ultimate level of LTR-directed gene expression is progressively diminished both in the induced and uninduced states by these deletions. Evidently the R and U3 region of the viral LTR encodes sequences important for high-level LTR-directed gene expression. Location of these sequences 3′ to the site of RNA initiation raises the possibility that they may be involved in post-transcriptional regulatory events as well as in contributing to the rate of RNA initiation. Sequences which have similar effects are reported to exist 3′ to the site of RNA initiation within the BLV LTR.

The second notable feature of the viral LTRs lies in asymmetry in the function of

the HTLV-I and -II sequences. The LTR of HTLV-I functions well as a promoter of heterologous genes in a wide variety of cell types, unrestricted as regards species or tissue of origin (Rosen et al. 1985). The activity of the HTLV-II LTR is markedly limited (Sodroski et al. 1985). It functions well in very few cell types. It is remarkable that the HTLV-II LTR does not function as a promoter in most human lymphoid cell lines, whether they be T or B cells. In fact, no promoter activity was observed in two human lymphoid cell lines that expressed a functional tat_{II} product (Sodroski et al. 1985a). The tat_{II} product in these cell lines was found to be capable of stimulating the HTLV-I LTR, while in the same cell lines no HTLV-II promoter activity was observed. It can be concluded that the HTLV-II promoter is either extremely fastidious as regards the requirement for cell-specified expression factors or that viral gene products of the *trans*-activator are required for activity of the HTLV-II LTR. Such other gene products cannot be supplied by the alternative reading frame product, pp27, as the HTLV-II LTR is inactive in the cell line that is reported to express both the HTLV-I *trans*-activator and pp27 proteins. The BLV LTR also displays a narrow cell line activity and is a very poor promoter in most uninfected cell types.

E. Transactivation: The pp27 and pp21 Proteins

A recent report by Inoue et al. (1986) indicates that the pp27 protein may play an important role in virus replication via a *trans*-acting mechanism. An integrated provirus deleted for the amino terminal portion of the *env* gene was found to be defective for RNA synthesis and for *gag* gene production. The deletion was such as to eliminate the 5′ coding exons of the *tat* and pp27 proteins. Transfection of a cell line containing this defective provirus with plasmids capable of expression of the *tat* and/or pp27 proteins revealed that *gag* gene synthesis was dependent upon both *tat* and pp27 gene expression from the transfected plasmids. Moreover, no *gag* gene RNA was detected upon transfection with the *tat* expressing plasmid alone.

This observation indicates that both the *tat* and pp27 proteins are needed for the expression of viral genes. Heterologous gene synthesis directed by the HTLV-I LTR, however, is not dependent on pp27, nor does the expression of pp27 markedly affect the rate of expression of such constructs (Rosen, Sodroski, Dokhelar, and Haseltine, unpublished observations).

The function of the pp27 gene resembles in a formal sense that of the *art* gene of HIV (Table 1). Neither pp27 nor *art* are required

Table 1

	None	tat_I	*pp27*	(tat_I+*pp27*)
LTR_I-heterologous gene	+	+ + + +	+	+ + + +
LTR_I-gag_I	−	−	−	+ + + +
LTR_I-tat_I	+	+	+	+
LTR_I-env_I		(unknown)		
LTR_{III}-heterologous gene	+	+	+	+
	None	tat_{III}	*art*	(tat_{III}+*art*)
LTR_{III}-heterologous gene	+	+ + +	+	+ + + +
LTR_{III}-gag_{III}	−	−	−	+ + + +
LTR_{II}-env_{III}	−	−	−	+ + + +
LTR_{III}-tat_{III}	+	+	+	+

LTR_I is the LTR of the HTLV-I virus.
LTR_{III} is the LTR of the HIV virus.
gag_I is the *gag* gene of the HTLV-I virus.
gag_{III} and env_{III} are the *gag* and *env* genes of HIV, respectively.
tat_I and tat_{II} are the *trans*-activators of the HTLV-I and HIV viruses, respectively.

for expression of heterologous genes under the control of the LTR. However, in the absence of a second gene product the *trans*-activator genes (*tat* genes) of these viruses are insufficient to permit expression of viral *gag* proteins. We nevertheless note that the *trans*-activator genes of both viruses can be synthesized in the absence of auxiliary proteins (Rosen, Sodroski, Dokhelar, and Haseltine, unpublished observations). For both viruses the regulatory genes are controlled independently from the structural genes.

Although the *art* and pp27 genes display a formal analogy in functional terms, such similarity does not necessarily imply that the mechanism of action is the same. The *trans*-activator gene of HTLV-I acts primarily as a transcriptional *trans*-activator of the viral LTR whereas the *trans*-activator of HIV is primarily a post-transcriptional activator. It remains to be tested whether the pp27 protein possesses an antirepression function as does *art,* although the preliminary genetics suggest that this is likely. Table 1 also shows that the *tat* genes of HTLV-I and of HIV do not reciprocally *trans*-activate the heterologous virus LTRs.

F. The Mechanism of Transformation

The process of in vitro formation of tumors by HTLV-I, -II, and BLV has not been fully characterized. Infection of T cells by the virus does not result in immediate tumor formation. Rather, tumors arise rarely (1 in 100–300 infected people over a lifetime). The role of viral genes in the transformation process is strongly inferred by epidemiological studies which link seropositivity to disease as well as the observation that T-cell tumors in infected people invariably contain at least one integrated copy of the provirus (Seiki et al. 1984). It is sometimes observed that tumors contain only the 3′ portion of the genome. However, most of the tumors found in patients contain, as a minimum, the 5′ LTR and the pX region.

T-cell tumors in patients are clonal with respect to the site of integration of the provirus (Seiki et al. 1983; Hahn et al. 1983). The long latent period and the clonal nature of the tumors indicate that events in addition to infection of T cells with the virus are required for the appearance of malignant tumors. Such events may represent either secondary changes occurring within the infected cell, such as somatic mutations, or changes in the immunological status of the host.

Two additional observations indicate that the viral genes play an important role in the initiation and maintenance of tumors. Tumorigenesis by the avian, murine, and feline retroviruses which contain only those genes required for virion formation and virus replication depend upon activation of cellular growth regulatory genes. This conclusion is reached from the observation that independent, virally induced tumors contain proviruses that are found integrated near the same cellular genes. Such is not the case for tumors induced by HTLV-I or BLV, for which no repeated chromosomal sites of integration have been observed in naturally occurring tumors (Seiki et al. 1983; Hahn et al. 1983). It is therefore inferred that viral genes themselves play a key role in the initiation and maintenance of the tumor phenotype.

The role of the viral genes in the transformation process is also inferred from in vitro transformation studies. Primary T cells can be immortalized by co-cultivation with infected cells treated with mitomycin C. In contrast to role cultures, recipient cell cultures continue to proliferate without continued antigen stimulation in the presence of the T-cell growth factor, IL-2. Eventually immortalized cells emerge from such cultures (Chen et al. 1983; Popovic et al. 1983; Miyoshi et al. 1981; Yamamoto et al. 1982). The expanding population of T cells is initially polyclonal with respect to the site of provirus integration. Cell lines that are monoclonal with respect to the sites of viral integration eventually emerge from the population and dominate the culture. Such cell lines may remain dependent upon IL-2 for growth or may become capable of IL-2 independent growth, depending on cell culture conditions. Such immortalized primary cells are typically $T4^+$ cells as are most HTLV-I induced tumors. $T8^+$ cell lines can be derived by co-cultivation of mitomycin-treated infected cells with primary populations of

lymphocytes enriched for cells which bear the T8 antigen (DeRossi et al. 1985).

It is possible that cell lines established from patient cells are not derived from tumors themselves but represent immortalization of the normal patient T cells by a mechanism analogous to that described for immortalization of T cells via co-cultivation. In this regard Waldmann and colleagues have found the T-cell receptor beta gene rearrangement in patient and tumor cells to differ (T. Waldmann, personal communication).

Events that occur between the initiation of infection and establishment of IL-2 dependent or independent T-cell lines have not been well characterized. Selection of specific fast growing clones may occur both in infected patients as well as in vitro. It is possible that secondary changes occur within the infected cell which permit rapid growth. Alternatively, the clonality of the tumor cells may represent selection of a cell population which expresses high levels of viral proteins that promote cellular growth.

G. Induction of the IL-2 Receptor by the *Trans*-activator Gene

The promoter of the IL-2 receptor and the IL-2 genes have been cloned. Cotransfection of the promoters placed 5′ to reported genes, such as the chloramphenicol acetylase transferase gene, with the *trans*-activator gene of HTLV-I has been shown to increase the level of expression of the IL-2 gene promoter (W. Greene, personal communication and our unpublished observations). The level of expression of the genes under the control of the IL-2 promoter was found be increased slightly in similar experiments. The *trans*-activator gene of HTLV-II has also been shown to increase the level of expression of the IL-2 receptor gene, albeit more weakly than that observed for the tat_I gene, at least in the particular experimental configuration used.

These observations suggest that the *trans*-activator gene of HTLV-II can contribute to the growth properties of the T cell by deregulation of genes which normally control T-cell proliferation in response to antigen stimulation. Such a model for T-cell transformation must include the additional consideration that the expression of the viral genes is dependent upon T-cell activation. Thus, an infected resting T cell should not be transformed as the viral genes are not expressed.

Although simple, this explanation for transformation does not suffice to account for the clinical observations with ATLL patients. If *tat* genes were sufficient to induce both IL-2 and IL-2 receptors, infection should lead to transformation. However, malignant growth of T cells in infected patients is a rare event. It is also possible that the pp27 and pp21 proteins play a role in the activation of cellular genes.

H. Summary

The broad outlines of mechanisms of tumorigenesis by the HTLV-I family of viruses are beginning to emerge. The viruses encode at least three genes in addition to the genes (*gag*, *pol*, and *env*) required for virus replication. These additional genes encoded for by the X region are likely to affect in a specific fashion the growth of lymphocytes. The *tat* gene appears to mimick at least part of the response of mature lymphocytes to recognition of the cognate antigen. That is, in T-lymphocytes the tat_I gene seems to induce the IL-2 and IL-2 receptor genes (W. Greene et al. 1986). The alternative reading-frame proteins, pp21 and pp27, have some similarity of cellular proteins that are associated with G_0 to G_1 transitions and may contribute to the transformed phenotype in cooperation with the *tat* gene.

The expression of viral genes in infected lymphocytes, the *tat* gene and pp21 and pp27 proteins, and possibly other viral genes (since the coding capacity of the X region is not exhausted by the *tat* and pp21 and pp27 proteins) may be sufficient to account for the transformation of T cells in culture. A secondary change in the infected cells in culture is not required to explain the outgrowth of cells which are clonal with respect to the site of viral genomic integration, as selection of the most rapidly growing infected cell could account for this observation.

The case of infected patients is more complex. Infection of T cells with the HTLV-I or

-II virus is not sufficient to produce malignant disease. Failure of the virus to induce malignancy in all infected T cells may be attributed to diverse causes. It is possible that viral gene expression is suppressed in most infected T cells. Certainly no viral RNA is detected in peripheral lymphocytes of infected patients which include the tumor cells themselves. Transcriptional repression of viral genes in infected cells is a sufficient explanation for the failure of the virus to transform most T cells in patients.

It is also possible that T cells which do express viral antigens are eliminated by the immune system. The observation that many tumor cell lines derived from patients contain deletions of virus structural proteins is consistent with this notion. Patients infected with HTLV-I and -II do show good immune responses to virion structural proteins.

An additional explanation may lie in homeostatic regulatory mechanisms of the immune response itself. Lymphocytes are thought to possess regulatory mechanisms that limit their proliferation response to antigen recognition. The early proliferative response of T cells in response to the presence of the cognate antigen is followed by reestablishment of a resting phase. Stabilization of the stimulated population of T cells was thought to involve activation of an internal cellular program of a repressive nature. Interaction of the activated T cells with other components of the immune system may also contribute to reestablishment of the resting state. It is conceivable that the homeostatic mechanisms regulating T-cell proliferation also regulate HTLV-I and -II gene expression and thereby limit the growth of infected cells in patients. In this view, malignant transformation by HTLV-I and -II requires bypass of the normal homeostatic mechanisms of growth control of lymphocytes. Such bypass may occur either by a secondary intracellular change that occurs in the infected cells or it may be due to a systemic failure of normal immunoregulatory mechanisms. Either process could give rise to a tumor cell population, the first by outgrowth of a cell which contains a secondary genetic lesion, and the second by overgrowth of the infected cell population by fast growing infected cells as is observed in cell culture.

The molecular biology and in vivo replication of the virus also provide some insight into the mechanisms of transmission into the virus. This family of viruses seems to be either poorly infectious or altogether noninfectious for uninfected cells. For establishment of infection it is likely that viral gene products transferred from an infected cell by cell fusion are required. The infectious unit may well be an infected cell rather than a cell previrion. In this context the X genes of this family of viruses are required for replication and may be viewed as replicative genes. Tumorigenesis may be a byproduct of the natural replicative cycle of this family of viruses.

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