The *TP53* gene encodes a nuclear protein implicated in the regulation of the cell cycle, DNA repair, and apoptosis. *TP53* mutations and other alterations have been described in numerous types of tumors, and some of these have been associated with poor prognosis. Some reports in the literature have indicated a relationship between *TP53* status and prognosis especially in non-small-cell lung cancer, breast cancer and NHL.

In order to characterize these molecular abnormalities and their clinical significance in prognosis, we also have analyzed the possible correlation between mutations in *TP53* gene, clinical findings, response to chemotherapy and survival in 49 children of our series. The mutations of *TP53* gene were analyzed by single-strand conformation polymorphism analysis (SSCP) of exons 5 through 9 and direct sequencing. Mutations of *TP53* were detected in 11 of 49 (22.5%) patients and more specifically in 20% of Burkitt's lymphoma. No significant correlation was found regarding age, gender, clinical stage and LDH level and *TP53* gene mutations. The comparison of EFS curves using the Log-Rank test were also not significant. However, the analysis of the effects of mutations on the core p53 structure identified biological and biochemical mutants with phenotypes probably related to different response to chemotherapy. Our data suggest that some types of mutants can alter the protein distinctly and may be associated with a more aggressive phenotype.

HTLV-1 $p12^{I}$ and $p30^{II}$ Proteins in Viral Persistence and Pathogenesis

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HTLV-1 is the only retrovirus known to be the etiologic agent of a human cancer, adult T cell leukemia/lymphoma (ATLL). HTLV-1 as well as some DNA viruses cause lifelong infections. In the case of DNA viruses, the inability of the host's immune response to clear virus-infected cells has been associated with viral latency and/or the ability of viral-encoded proteins to interfere with the host's immune response to the virus, particularly to evade CTLs, which play a major role in cellular immunity against cell-associated viruses.

We hypothesized that this may be the case also for HTLV-1 and focused on both the $p12^{I}$ and $p30^{II}$ proteins encoded by alternatively spliced mRNAs from ORFs I and II, respectively, within the 3' end of the viral genome. The HTLV-1 $p12^{I}$ protein localizes to the ER and Golgi, exhibits weak oncogenic activity, shares aa similarities with the bovine papillomavirus type 1 E5 oncoprotein, and binds to the IL-2R $ and ($_c$ chains. The spliced mRNA encoding $p12^{I}$ has been detected in *in vitro* and *ex vivo* HTLV-1-infected T cells and macrophages. Sera from rabbits experimentally infected with HTLV-1, as well as sera from humans infected with HTLV-1, have been shown to recognize the ORF I product , and a CTL response to ORF I products can be detected in HTLV-1-infected individuals. Ectopic expression in Hela-Tat cells or overexpression of $p12^{I}$ in PBMCs is associated with enhancement of STAT5 activation and decreased IL-2 requirement for proliferation. This effect however is strictly dependent on activation of T cells by both ligation of TCR and PHA stimulation and is not observed in HTLV-1-immortalized T cells since culture conditions (IL-2 addition) likely compensate for ORF I expression. The work of others has demonstrated that, in the presence of PMA, $p12^{I}$ induces calcium release and NFAT activation and transcriptional modulation of cellular genes. A seminal finding was that the ablation of $p12^{I}$ in an infectious clone impairs viral infectivity of primary lymphocytes *in vitro* and, importantly, in rabbits *in vivo*. A recent report suggested that $p12^{I}$ may not be essential for viral transmission. However, this conclusion was drawn from the finding of *in vivo* $p12^{I}$ mutations that preserve 84% of the $p12^{I}$ protein. The HTLV-1 $p30^{II}$ is a nuclear/nucleolar protein [5] and contains a highly conserved bipartite nuclear localization signal (NLS) between aa 71 and 98, which can be functionally substituted for the NLS of Rex. In addition, $p30^{II}$ contains serine- and threonine-rich regions that share distant homology to the activation domain of transcriptional activators, such as Oct-1/2, Pit-1, and POU-1, that modulates cell gene expression and is important for *in vivo* infectivity. Thus, both $p12^{I}$ and $p30^{II}$

play important roles in viral infectivity *in vivo*. There is the need for further investigation on which functions of $p12^{I}$ and $p30^{II}$ contribute to impaired infectivity *in vivo* as these proteins may play a key role in viral persistence and mitotic propagation of the virus (reviewed in X).

$p12^{I}$ binds to the newly synthesized MHC class I heavy chain (MHC class I-Hc) before its association with $\$_2$-microglobulin and targets it for degradation by the proteasome. As a result of the Hc-$p12^{I}$ interaction, there is a disruption of intracellular trafficking of the MHC class I complex and a significant decrease in MHC class I at the surface of human T cells. We hypothesized that decreased density of MHC class I-viral peptide complexes on the cell surface may impair recognition of HTLV-1-infected cells by CTLs. To test this hypothesis, a relevant system composed of B cells from an MHC class I A2-positive individual and $CD8^{+}$ CTL clones able to recognize the Tax peptide presented by the MHC class I A2.01 was used. Both $p12^{I}$ and Tax can be expressed in the B cell line as demonstrated by the dose-dependent increase of Tax-induced transactivation of an HTLV-1 LTR-Luc construct. As expected, the expression of $p12^{I}$ was associated with decreased MHC class I A2 expression on the surface of the human B cells. Cytolytic activity on targeted B cells was observed when more than 0.5 mg of Tax were transfected and $p12^{I}$ inhibited CTL activity in a dose-dependent manner, demonstrating that $p12^{I}$ expression can result in CTL killing of human target cells expressing Tax.

Others have shown that $p12^{I}$ increases calcium release and NFAT activation upon stimulation of T cells with PMA. This effect bypasses antigen receptor ligation, as $p12^{I}$ increases PMA-induced NFAT activation also in LAT-deficient T cells. Following ligation of TCR by antigen or Abs, TCR signaling occurs through a cascade of events that involves, at first, phosphorylation of protein tyrosine kinase, Lck, and Fyn that, in turn, phosphorylate the tyrosines in the cytosolic domain of TCRz and CD3, which become the docking sites for ZAP70. Activated ZAP70 phosphorylates LAT that binds Grb2, PLC-g1, and the p85 subunit of phosphatidylinositol 3-kinase and probably indirectly Vav, Cb1, and SLP76. The recruitment of these critical molecules to the membrane ultimately causes calcium release, dephosphorylation and nuclear translocation of NFAT, enhancement of transcription, cytokine production, and T cell proliferation. Interestingly, while PMA stimulation of T cells increases NFAT activity, which is enhanced by $p12^{I}$-induced calcium release, we observed that, following ligation of the TCR with aCD3e alone, expression of $p12^{I}$ in Jurkat T cells resulted in a decrease in NFAT activity. In the same experiment, PMA stimulation of cells resulted in increased NFAT activity, as previously described. Both the negative effect of $p12^{I}$ on TCR signaling and the positive effect following PMA stimulation were dose-dependent. Costimulation with the aCD28 Ab did not restore downregulation of TCR signaling. To investigate the mechanism of $p12^{I}$-induced downregulation of NFAT activity, we assessed phosphorylation of cellular proteins following ligation of the TCR. A decreased phosphorylation of several cellular proteins in the range of 100 to 210 kDa was found, and, among them, PLC-g1, Vav, and Cbl; ZAP70, however, was not affected, consistent with a possible effect of $p12^{I}$ on LAT. Indeed, we found that $p12^{I}$ binds preferentially to nonphosphorylated LAT. As LAT resides in the rafts [30], we assessed whether $p12^{I}$ is also localized to the lipid rafts. Flotation assay demonstrated that $p12^{I}$ is enriched in fractions containing Lck, a raft-associated protein in Jurkat T cells. Similar results were obtained when $p12^{I}$ was expressed in 293T cells whereby $p12^{I}$ colocalized with Lyn, a protein found in the raft. To confirm by an independent approach $p12^{I}$ raft localization, $p12^{I}$ was expressed in Jurkat T cells, and these were stained with FITC-conjugated cholera toxin (Co-Tx) (FITC-Co-Tx) that binds to the raft [31]. The Ab to $p12^{I}$ stained a subset of cells that were also costained by FITC-Co-Tx, and image merging demonstrated colocalization of $p12^{I}$ and Co-Tx. Thus, $p12^{I}$ localizes not only to the ER-Golgi but also in lipid microdomains. Antigen-presenting cells (APCs), such as B cells, transduce signals to T cells through the TCR. The TCR located in the rafts translocates to the central core of the T cell supramolecular activation cluster within the cell-cell junction termed the immunological synapse (IS). Because $p12^{I}$ is found in the rafts and affects TCR signaling, we hypothesized that $p12^{I}$ may be recruited in the IS. To test this, Jurkat T cells and Raji cells were used as an *in vitro* model of T cell and APC

interaction that involved the TCR and MHC class II. Indeed, polarization of the TCR was seen within 15 min of cocultivation of Jurkat T cells with superantigen staphylococcal enterotoxin E (SEE)-prepulsed Raji B cells. In the same experimental conditions, within 15 min, the $p12^{I}$ protein expressed in Jurkat T cells coactivated with SEE-prepulsed Raji B cells, polarized together with CD3e to the IS. IS enriched for $p12^{I}$ was also observed at 30 min from cocultivation. As expected, IS formation was not observed in the absence of SEE.

HTLV-1-Encoded $p30^{II}$ is a Post-Transcriptional Negative Regulator of Viral Replication

The stoichiometry and the catalytic activity of Tax, the viral transactivator, determine T cell progression through the G1 phase of the cell cycle (reviewed in X). The Rex protein promotes viral production by regulating the transport of genomic and envelope viral mRNAs to the cytoplasm and influences the expression of other cellular genes (reviewed in X). Because Tax is highly immunogenic, it was hypothesized that HTLV-1 in addition to interfering with CTL recognition through $p12^{I}$ may have evolved a dedicated genetic function to reduce the expression of viral proteins (including Tax) and become transiently dormant. This would help the virus-infected cells evade host immune surveillance, a strategy commonly used by DNA viruses. To this end, the effect on viral replication of the HTLV-1 $p30^{II}$ protein, encoded by the doubly spliced mRNA from ORF II, was investigated. $p30^{II}$ was chosen because the nuclear and nucleolar localization of this protein suggested that it might have regulatory function(s). We demonstrated indeed that HTLV-1 has evolved a genetic function to restrict its own expression by a novel post-transcriptional mechanism. The HTLV-1-encoded $p30^{II}$ is a nuclear-resident protein that binds to and retains in the nucleus the doubly spliced mRNA encoding the Tax and Rex proteins. Because Tax and Rex are positive regulators of viral gene expression, their inhibition by $p30^{II}$ reduces virion production, and this is true also in human T cell lines HUT102, C91PL, and MT2 chronically infected with HTLV-1. In a collaborative study with P.L. Green and M.D. Lairmore, we also showed that the HTLV-1 $p30^{II}$ represses by a post-transcriptional mechanism HTLV-2 expression and that HTLV-2 also encodes a protein ($p28^{II}$) able to suppress both HTLV-2 and HTLV-1 replication.

The HTLV-1 $p30^{II}$ has a negative effect on viral replication. Therefore, it could be thought of as a "latency protein." Mechanistic studies on $p30^{II}$ function and regulation and whether inhibition of $p30^{II}$ function may reveal hidden infected cells to host immune surveillance are subjects that need further investigation. Our demonstration that $p30^{II}$ binds to the splice junction for the Tax/Rex mRNA and not to the $p21^{Rex}$ RNA demonstrates specificity. However, the precise nature of this interaction has not been unveiled.

National Cancer Institute Initiatives for International and AIDS-Related Cancer Research

Jodi B. Black

The global burden of cancer is large and projected to grow larger. Each year there are approximately 10 million new cancer cases and more than 6 million deaths worldwide. In many developed countries, including the United States, cancer accounts for more than 20% of all deaths. Although all-site cancer rates are generally lower in less developed countries, these regions are projected to incur the most rapid increase in cancer rates over the next few decades. Cancer in developing countries is expected to represent 70% of the global cancer burden by 2030. Developing countries also bear the majority of the global burden of HIV infections and associated co-morbid conditions including cancer. Thus, the National Cancer Institute (NCI) supports cancer and AIDS related malignancy research outside the United States by highly qualified foreign nationals. In addition, the NCI supports collaborative research involving US and foreign investigators and the training of US scientists abroad and foreign scientists in the US.