Meeting Report on the 13th International Conference on Human Retrovirology: Human T-Cell Leukemia Virus Research

30 Years after Adult T-Cell Leukemia

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Introduction

Thirty years ago, in 1977, a new clinical entity called adult T-cell leukemia (ATL) was described in Japan by Takatsuki and colleagues. Subsequently, Poiesz, Ruscetti, Gallo, and coworkers; and Yoshida, Miyoshi, Hinuma, and colleagues isolated a human retrovirus named human T-cell leukemia virus type-1 (HTLV-1). The history of the discovery of ATL and the isolation of HTLV-1 has been recently reviewed elsewhere (1–3).

From May 22 to 25, 2007, 350 researchers gathered in Hakone, Japan, at the 13th International Conference on Human Retrovirology organized by Toshiki Watanabe (University of Tokyo, Tokyo, Japan) to discuss the latest finding on HTLV-1 pathogenesis. The meeting opened with a keynote presentation by Kuan-Teh Jeang [National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD] reviewing cell proliferative changes, genetic damaging events, and check point inactivation in the development of ATL (4). Over the next 4 days, >320 papers were presented. Conference highlights are summarized below.

Epidemiology of HTLV

HTLV infects 10 to 20 million individuals worldwide. Masahiro Satake (Tokyo Red Cross Blood Center, Tokyo, Japan) reported on HTLV-1 positivity rate (0.37%) in Japanese first-time blood donors. HTLV-1 seropositivity in donors was 1.18% in their 30s in 1995 and 1.28% in their 40s in 2005, showing an 8% increase in 10 years. The same trend was found in the donors who were an additional 10 years older. Satake interpreted these figures to reveal the rate of horizontal infection in Japan. How individuals become susceptible to HTLV infection was commented upon by Sabine Plancoulaine [Institut National de la Sante et de la Recherche Medicale (INSERM), Paris, France] using a genome-wide linkage analysis to HTLV infection was commented upon by Sabine Plancoulaine [Institut National de la Sante et de la Recherche Medicale (INSERM), Paris, France] using a genome-wide linkage analysis mapping the susceptibility locus in children of African origin for HTLV-1 infection through breast feeding. She found significant linkage between HTLV-1 susceptibility and two chromosomal loci at 2p25 and 6q27.

Note: The 13th International Conference on Human Retrovirology was held from May 22–25, 2007 in Hakone, Japan.

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HTLV-1 infection is also associated with an inflammatory pathology termed HTLV-associated myelopathy (HAM) or tropical spastic paraparesis (TSP). The association of neurologic abnormalities and HTLV-1 infection was discussed by Edward Murphy (University of California San Francisco, San Francisco, CA). In his study (HTLV Outcome Study), Murphy followed 151 HTLV-1–positive and 387 HTLV-2–positive former blood donors for 10 years (5). Compared with uninfected controls, HTLV-1-positive subjects had significantly more motor and coordination abnormalities. Murphy concluded that HTLV-1 and HTLV-2 infections are associated with a spectrum of neurologic defects beyond those described previously for classic HAM.

Tax, Transcription, Cellular Proliferation, and Transformation

The HTLV-1 Tax oncoprotein induces cellular proliferation, survival, and genetic damage. Nuclear factor-κB (NF-κB) has been described as an important cellular target activated by Tax through both the canonical and noncanonical pathways. Masahiro Fujii (Niigata University, Niigata, Japan) suggested in new findings a specificity of noncanonical NF-κB activation in HTLV-1 pathogenesis. He found that noncanonical activation of NF-κB, as measured by p52 processing from its p100 precursor, is restricted to HTLV-1, but not HTLV-2. Tax. Interestingly, HTLV-1, but not HTLV-2, is oncogenic. Fujii further reported that noncanonical NF-κB activation depends on the PDZ binding motif (PBM) of Tax1 (located in carboxyterminal amino acids 350–353).

Following-up on the role of Tax PBM, complete genome sequences from two recently isolated African HTLVIs, HTLV-3 (Sara Calattini and Renaud Mahieux, Pasteur, Paris, France) and HTLV-4 (William Switzer, Centers for Disease Control and Prevention, Atlanta, GA), were presented. The data revealed that HTLV-3 Tax retains a PBM that is absent from HTLV-4 Tax. This observation predicts different pathogenic outcomes from HTLV-3 and HTLV-4 infections.

Two presentations addressed Tax and genetic instability. Susan Marriott (Baylor College of Medicine, Houston, TX) reported that Tax represses double-strand DNA repair, which is partly governed by a protein complex containing DNA-protein kinase (DNA-PK). John Semmes (Eastern Virginia University, Norfolk, VA) showed that Tax binds DNA-PK directly and sequesters this factor into nuclear speckles, thus repressing double-strand DNA repair.
Tax can affect host cell metabolism through transcriptional activation of cellular genes. Jennifer Nyborg (Colorado State University, Fort Collins, CO) found that Tax promotes cyclic AMP–responsive element binding protein (CREB) phosphorylation, increasing the availability of modified CREB for promoter transactivation. Paul Laybourn (Colorado State University) identified a new function of Tax abrogation of H1-mediated repression of p300 coactivator activity. Laybourn also found that Tax decreased histone mRNA levels, arguing that such reduction may contribute to dysregulated expression of many cellular genes. Additional insight into Tax activation of promoters was provided by Nicholas Polakowski and Isabelle Lemonass (East Carolina University, Greenville, NC) who used a chromatin immunoprecipitation assay/microarray (ChIP-on-CHIP) approach to describe direct recruitment of Tax to many cellular promoters.

What are some of these promoters? Cynthia Pise-Masison’s [National Cancer Institute (NCI), Bethesda, MD] microarray analyses revealed increased expression in ATL leukemic cells of genes linked to cell cycle progression (e.g., CDK2, CCN1), apoptosis repression (e.g., survivin), and tyrosine kinases (e.g., Lyn). Separately, Ralph Grassmann (University of Erlangen-Nuremberg, Erlangen, Germany) and Kuan-Teh Jeang (NIH) showed altered microRNA (miRNA) expression in HTLV-1–transformed cells, suggesting that Tax also affects/occupies promoters that transcribe oncogenic miRNAs.

Many questions pertaining to in vivo Tax and virus expression are currently unanswered. Charles Bangham (Imperial College, London, United Kingdom) used deuterated glucose for labeling patients’ lymphocytes to check the in vivo effect of Tax/HTLV expression on lymphocyte population dynamics. He saw increased proliferation of CD4+CD45RO+ and CD8+CD45RO+ T-lymphocytes in HTLV-1–infected persons compared with controls, with the additional production of 10^5 lymphocytes per year in the former. The in vivo proliferation rate of CD4+CD45RO+ cells correlated with tax expression (measured ex vivo), supporting a physiologic role (directly or indirectly) for this viral oncoprotein in the proliferation of infected cells (6).

Functional Contributions from HTLV Accessory Genes

Besides gag, pol, env, and tax, HTLV encodes rex and accessory genes hbz, p12, p13, p27, and p30. Agnes Lezin (Fort-De-France, Martinique) quantified the expression levels of the HTLV-1 accessory genes in primary cells from HAM/TSP, ATL, and asymptomatic patients. She reported that that p12, p30, and p30 expression was undetectable whereas levels of tax, hbz, and p13 transcripts quantitatively correlated with clinical progression.

HTLV-1 HBZ is unique among viral proteins in being encoded by the minus strand of the provirus. Published studies suggested that hbz can function as an RNA and as a protein. At this meeting, Masao Matsouka (Kyoto University, Kyoto, Japan) mapped HBZ proliferative activity to the 5′ end of its RNA. His transgenic mice constructed to express HBZ gene driven by the mouse CD4 promoter/enhancer showed increased CD4⁺ T cells in the spleen with infiltration into the skin and lung. Osamu Isono (Kyoto University) presented evidence that HBZ protein represses c-Jun activity by destabilizing c-Jun and promoting its proteosomal degradation. Jean-Michel Mesnard (Centre National de la Recherche Scientifique, Montpellier, France) had previously published that HBZ protein stimulates JunD activity. Following up on that finding, Sophie Kuhlmann (INSERM, Lyon, France) reported that HBZ/JunD interaction up-regulates expression of the human telomerase reverse transcriptase gene, potentially explaining elevated telomerase activity in ATL cells.

The p30 protein promotes replication and persistence of HTLV-1 by acting at transcriptional and posttranscriptional levels. Vefa Franchini (NCI) used an infectious proviral clone to show the action of p30 in posttranscriptional gene repression. She showed that deletion of p30 from the provirus increased Tax expression and HTLV-1 replication. Michael Lairmore (Ohio State University, Columbus, OH) followed with data that added complexity to p30 function. He found increased phosphorylation of cell cycle regulators Cdc25C and Chk1 in Jurkat T-cells expressing p30, and p30 association with nuclear γ-H2AX DNA-repair foci. Lairmore proposed a role for p30 in signaling DNA damage (perhaps triggered by retroviral integration).

HTLV-1 p13 and Rex were also discussed at this meeting. Vincenzo Ciminale showed that p13 expression increased mitochondrial reactive oxygen species, influencing the metabolism of the cell by favoring aerobic metabolism, and moderating apoptosis. Patrick Green (Ohio State University) discussed that the phosphorylation status of HTLV-2 Rex at its COOH terminus is important for protein stability and function. Phosphomimetic Rex mutants were shown to be constitutively active, and these Rex mutant viruses displayed increased viral replication both in vitro and in inoculated rabbits. However, Rex mutant viruses were similar to wild-type virus in the capacity to immortalize T lymphocytes in culture and persist in infected rabbits.

Immunologic Implications of Viral Infection

Inflammatory lesions in HAM/TSP are associated with the breakdown of the blood-brain barrier. The factors that cause the breakdown of the blood-brain barrier are not known. Philippe Afonso (Pasteur Institute, Paris, France) showed that HTLV-1–infected lymphocytes could induce an increase in both paracellular endothelial permeability and transcellular migration in an in vitro model of the blood-brain barrier. The observed changes were accompanied by disruption of intercellular tight junctions and seemed to be mediated by interleukin (IL)–1 and tumor necrosis factor-α.

HTLV-1 Tax induces strong expression of the high-affinity IL-2 receptor chain (CD25) on the infected CD4⁺ T cell. CD25 is also characteristically expressed at a high level on the malignantly transformed CD4⁺ T cells in ATL. These observations have stimulated considerable interest because certain CD4⁺ CD25⁺ T cells, known as regulatory T cells (Treg), can suppress the proliferation and function of other T cells. It is now becoming clear that the intracellular expression of the transcriptional factor FoxP3 in CD4⁺ T cells is more strongly associated with a Treg phenotype than is CD25 expression, because CD25 is also characteristically expressed on activated T cells. Because of the expression of both CD25 and, in a proportion of cases, FoxP3 in ATL cells, questions have recently been raised whether HTLV-1 preferentially infects or transforms CD4⁺ CD25⁺ FoxP3⁺ T cells (Shimeru Kamihira, Nagasaki University, Nagasaki, Japan) and whether the ATL cells themselves act as Treg. The latter
is an important possibility because ATL is characterized by even more profound immunosuppression than is seen in other leukemias.

Hiroki Yano (Nagoya City University, Nagoya, Japan) tested the ability of ATL cells (identified by a receptor frequently coexpressed on ATL cells, CCR4) to inhibit the proliferation of autologous CD4+ non-ATL cells. In five ATL cases studied, evidence was obtained of a degree of suppression in one case, and suggestive evidence was obtained in one further case. Frederic Toulza (Imperial College, London, United Kingdom) used a simple phenotypic definition of Treg cells, CD4+ FoxP3+, and reported that the percentage of CD4+ FoxP3+ cells in the circulation was negatively correlated with the rate of lysis of autologous naturally infected CD4+ T cells by the patient’s CD8+ CTLs. The results suggest that CD4+ FoxP3+ cells play a major role in determining the efficiency of CTL surveillance of HTLV-1. Because there is increasing evidence that the CTL response to this virus determines the proviral load and the risk of inflammatory disease, it is possible that Treg are an important determinant of the outcome of HTLV-1 infection.

Mari Kannagi (Tokyo Medical and Dental University, Tokyo, Japan) reported that immunization of orally infected rats with syngeneic, mitomycin C–treated HTLV-1–transformed cells boosted the cellular immune response to HTLV-1, and that this strengthened immune response was accompanied by a reduction in the proviral load (7). Charles Bangham has evidence for abnormally rapid turnover in vivo of both HTLV-1–infected CD4+ T cells and CD8+ CTLs (which include the HTLV-1–specific CTLs; ref. 6) with a typical HTLV-1–infected individual turning over an extra 10^{12} lymphocytes per year. Thus, the findings from rats (7) and humans (6) lend weight to the turning over an extra 10^{12} lymphocytes per year. Thus, the findings from rats (7) and humans (6) lend weight to the

Therapeutic Approaches to HTLV-1 Infection

Several speakers reviewed various ATL treatment options. Deidre O’Mahony (NCI) presented a phase 1 trial of Splizumab, a monoclonal antibody that binds to CD2 on human T-cells and natural killer cells, in CD2-positive lymphoid malignancies, including aggressive ATL with early encouraging response rates and relatively low toxicities. Lee Ratner (Washington University, St. Louis, MO) reported mixed clinical and virological findings from etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) infusional chemotherapy followed by consolidation and maintenance in 67 patients who received RIST, 14 patients achieved complete remission. Three-year overall survival and event-free survival were 67 ± 12% and 55 ± 12%, respectively. Further explaining why stem cell transplantation is effective for ATL, Nanae Harashima (Tokyo Medical and Dental University) reported that Tax-specific CTL response was recovered [by stimulating peripheral blood mononuclear cells (PBMC) in vitro with HTLV-1–infected cells] after stem cell transplantation, suggesting a role for CTL in eliminating Tax-expressing cells and preventing relapse of ATL.

Seishi Ogawa and Toshiki Watanabe (University of Tokyo) described molecular allelo-karyotyping of primary ATL cells using single-nucleotide polymorphism genotyping microarrays. ATL lymphoma-type genomes from 108 patients showed characteristic copy number profiles and unique patterns of allelic imbalances. Future detailed molecular investigation of these results could reveal novel drug targets for ATL treatment.

For TSP/HAM treatment, Agnès Lézin (Fort De France, Martinique) is investigating the use of histone deacetylase inhibitors, valproate, to activate the expression of latent HTLV-1 proviruses in vivo and expose the activated infected cells to CTL immune surveillance. Lézin reported that valproate induced a significant reduction of provirus loads (mean 24-fold) in HAM/TSP patients.

Development of Mouse Models for HTLV-1 and ATL

To date, oncogenic potential of Tax in transgenic mice has been intensively studied. However, all extant lymphoid and nonlymphoid tumors in mice have lacked the CD4 surface marker. By contrast, CD4 is present on almost all patient ATL cells. Hideki Hasegawa (National Institute of Infectious Diseases, Tokyo, Japan) had published recently that transgenic mice expressing Tax driven by a lymphocyte-specific protein tyrosine kinase Lck-proximal promoter, targeting immature thymocytes, developed CD4+ CD8− double-negative T-cell leukemia/lymphoma (8). In new findings using the distal Lck promoter, Takeo Ohsugi and Toru Urano (Kumamoto University, Kumamoto, Japan) now reported a tax transgenic mouse, which developed CD4+ T-cell leukemia (9).

It is popular to use nonobese diabetic severe combined immunodeficient (NOD-SCID) common γ-chain null (NOG) mice for transplantation with human cells. Paola Miyazato (Kyoto University) showed that spread of HTLV-1 infection in human PBMCs transplanted into NOG mice could be blocked by azidothymidine and tenofovir but only in early stages of infection. She discussed that Tax mRNA expression in the infected human cells in NOG mice increased after in vitro culture, similar to that seen in human cases. In a related approach, Prabal Banerjee (State University of New York, Syracuse, NY) explanted HTLV-1–infected human CD34+ hematopoietic progenitor cells into NOD-SCID mice. He observed HTLV-1–infected human T cells, B cells, and monocytes/macrophages in transplanted mice. Some of the mice showed proliferation of HTLV-1–infected CD4+ T cell in lymphoid tissues as well as HTLV-1–infected CD34+ cells persisting in the bone marrow and thymus. Banerjee also reported that reconstitution of NOD-SCID mice with HTLV-1 Tax-transduced human CD34+ cells produced proliferating CD4+ T cells.

Future Perspectives

In the 30 years since the description of ATL, much has been learnt about HTLV-1 pathogenesis. Going forward, several important questions remain to be addressed for oncological research on ATL. Will the next years bring clarifying insights into roles contributed by human miRNAs to ATL initiation and progression? Will we learn...
why Tax is dispensable for ATL maintenance and why ATL does not use the "oncogene addiction" model of transformation? Will findings of virus-induced senescence, checkpoint inactivation, and cellular genetic damage meld into a coherent chronology of transformation events? In addition to these questions, equally important topics challenge immunologic and clinical HTLV-1 research. Indeed, one can look with anticipation to many intriguing answers that await revelation over the next 30 years.

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**References**
